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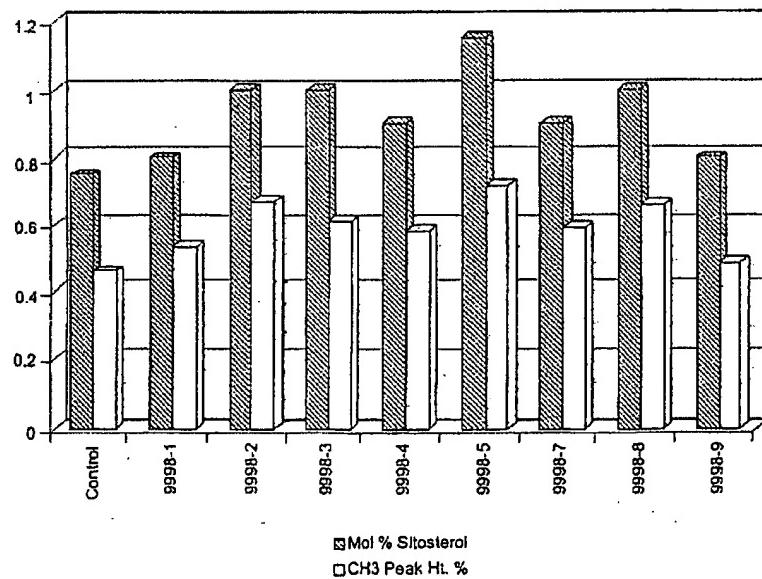
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(54) Title: PLANT STEROL ACYLTRANSFERASES



(57) Abstract: The present invention is directed to lecithin: cholesterol acyltransferase-like polypeptides (LCAT) and acyl CoA: cholesterol acyltransferases-like polypeptides (ACAT). The invention provides polynucleotides encoding such cholesterol: acyltransferases-like polypeptides, polypeptides encoded by such polynucleotides, and the use of such polynucleotides to alter sterol composition and oil production in plants and host cells. Also provided are oils produced by the plants and host cells containing the polynucleotides and food products, nutritional supplements, and pharmaceutical composition containing plants or oils of the present invention. The polynucleotides of the present invention include those derived from plant sources.

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## PLANT STEROL ACYLTRANSFERASES

### CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. provisional application Serial No. 60/152,493, filed August 30, 1999 and herein incorporated by reference in its entirety for all purposes.

### BACKGROUND

10 Technical Field

The present invention is directed to plant acyltransferase-like nucleic acid and amino acid sequences and constructs, and methods related to their use in altering sterol composition and/or content, and oil composition and/or content in host cells and plants.

15 Related Art

Through the development of plant genetic engineering techniques, it is now possible to produce transgenic varieties of plant species to provide plants which have novel and desirable characteristics. For example, it is now possible to genetically engineer plants for tolerance to environmental stresses, such as resistance to pathogens and tolerance to 20 herbicides. It is also possible to improve the nutritional characteristics of the plant, for example to provide improved fatty acid, carotenoid, sterol and tocopherol compositions. However, the number of useful nucleotide sequences for the engineering of such characteristics is thus far limited.

There is a need for improved means to obtain or manipulate compositions of sterols 25 from biosynthetic or natural plant sources. The ability to increase sterol production or alter the sterol compositions in plants may provide for novel sources of sterols for use in human and animal nutrition.

Sterol biosynthesis branches from the farnesyl diphosphate intermediate in the isoprenoid pathway. Sterol biosynthesis occurs via a mevalonate dependent pathway in 30 mammals and higher plants (Goodwin,(1981) *Biosynthesis of Isoprenoid Compounds*, vol 1 (Porter, J.W. & Spurgeon, S.L., eds) pp.443-480, John Wiley and Sons, New York), while in green algae sterol biosynthesis is thought to occur via a mevalonate independent pathway (Schwender, et al. (1997) *Physiology, Biochemistry, and Molecular Biology of*

*Plant Lipids*, (Williams, J.P., Khan, M.U., and Lem, N.W., eds) pp. 180-182, Kluwer Academic Publishers, Norwell, MA).

The solubility characteristics of sterol esters suggests that this is the storage form of sterols (Chang, *et al.*, (1997) *Annu. Rev. Biochem.*, 66:613-638). Sterol O-acyltransferase enzymes such as acyl CoA:cholesterol acyltransferase (ACAT) and lecithin:cholesterol acyltransferase (LCAT) catalyze the formation of cholesterol esters, and thus are key to controlling the intracellular cholesterol storage. In yeast, it has been reported that overexpression of *LRO1*, a homolog of human LCAT, and phospholipid:diacylglycerol acyltransferase increased lipid synthesis (Oelkers *et al.*, (2000) *J. Biol. Chem.*, 26:15609-15612; Dahlqvist *et al.*, (2000) *Proc. Natl. Acad. Sci. USA*, 97:6487-6492).

The characterization of various acyltransferase proteins is useful for the further study of plant sterol synthesis systems and for the development of novel and/or alternative sterol sources. Studies of plant mechanisms may provide means to further enhance, control, modify, or otherwise alter the sterol composition of plant cells. Furthermore, such alterations in sterol content and/or composition may provide a means for obtaining tolerance to stress and insect damage. Of particular interest are the nucleic acid sequences of genes encoding proteins which may be useful for applications in genetic engineering.

#### SUMMARY OF THE INVENTION

The present invention is directed to lecithin:cholesterol acyltransferase-like polypeptides (also referred to herein as LCAT) and acyl CoA:cholesterol acyltransferase-like polypeptides (also referred to herein as ACAT). In particular the invention is related to polynucleotides encoding such sterol:acyltransferases, polypeptides encoded by such polynucleotides, and the use of such polynucleotides to alter sterol composition and oil production. The polynucleotides of the present invention include those derived from plant sources.

One aspect of the invention, therefore, is an isolated nucleic acid sequence encoding a plant lecithin:cholesterol acyltransferase-like polypeptide, a fragment of a plant lecithin:cholesterol acyltransferase-like polypeptide, a plant acyl CoA:cholesterol acyltransferase-like polypeptide or a fragment of a plant acyl CoA:cholesterol acyltransferase-like polypeptide.

Another aspect provides an isolated nucleic acid sequence consisting essentially of SEQ ID NO: 2, 4, 6, 8, 10-29, 43-51, 73 or 75. Also provided is an isolated nucleic acid sequence consisting of SEQ ID NO: 2, 4, 6, 8, 10-29, 43-51, 73 or 75.

- Still another aspect provides an isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 3 or SEQ ID NO: 3 with at least one conservative amino acid substitution; SEQ ID NO: 2; an isolated polynucleotide that has at least 70%, 80%, 90%,  
5 or 95% sequence identity with SEQ ID NO: 2; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 2; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 2 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.
- 10 Still another aspect provides an isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3' where X is a hydrogen, Y is a hydrogen or a metal, R<sub>1</sub> and R<sub>2</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>3</sub> is selected from the group consisting of an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 3 or SEQ ID NO: 3 with at least one conservative  
15 amino acid substitution; SEQ ID NO: 2; an isolated polynucleotide that has at least 70%, 80%, 90%, or 95% sequence identity with SEQ ID NO: 2; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 2; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 2 and encodes a plant  
20 lecithin:cholesterol acyltransferase-like polypeptide.
- Another aspect provides an isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of an isolated polynucleotide encoding a polypeptide of SEQ ID NO:5 or SEQ ID NO: 5 with at least one conservative amino acid substitution; SEQ ID NO: 4; an isolated polynucleotide that has at least 70%, 80%, 90%,  
25 or 95% sequence identity with SEQ ID NO: 4; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 4; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 4 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.
- 30 Another aspect provides an isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3' where X is a hydrogen, Y is a hydrogen or a metal, R<sub>1</sub> and R<sub>2</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>3</sub> is selected from the group consisting of an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 5 or SEQ ID NO: 5 with at least one conservative amino acid

substitution; SEQ ID NO: 4; an isolated polynucleotide that has at least 70%, 80%, 90%, or 95% sequence identity with SEQ ID NO: 4; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 4; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that 5 hybridizes under stringent conditions to SEQ ID NO: 4 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.

Another aspect provides an isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 7 or SEQ ID NO: 7 with at least one conservative amino acid 10 substitution; SEQ ID NO: 6; an isolated polynucleotide that has at least 70%, 80%, 90%, or 95% sequence identity with SEQ ID NO: 6; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 6; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 6 and encodes a plant 15 lecithin:cholesterol acyltransferase-like polypeptide.

Another aspect provides an isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3' where X is a hydrogen, Y is a hydrogen or a metal, R<sub>1</sub> and R<sub>2</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>3</sub> is selected from the group consisting of an isolated polynucleotide encoding a 20 polypeptide of SEQ ID NO: 7 or SEQ ID NO: 7 with at least one conservative amino acid substitution; SEQ ID NO: 6; an isolated polynucleotide that has at least 70%, 80%, 90%, or 95% sequence identity with SEQ ID NO: 6; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 6; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that 25 hybridizes under stringent conditions to SEQ ID NO: 6 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.

Another aspect provides an isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 9 or SEQ ID NO: 9 with at least one conservative amino acid 30 substitution; SEQ ID NO: 8; an isolated polynucleotide that has at least 70%, 80%, 90%, or 95% sequence identity with SEQ ID NO: 8; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 8; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that

hybridizes under stringent conditions to SEQ ID NO: 8 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.

- Another aspect provides an isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3' where X is a hydrogen, Y is a hydrogen or a metal, R<sub>1</sub> and R<sub>2</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>3</sub> is selected from the group consisting of an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 9 or SEQ ID NO: 9 with at least one conservative amino acid substitution; SEQ ID NO: 8; an isolated polynucleotide that has at least 70%, 80%, 90%, or 95% sequence identity with SEQ ID NO: 8; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 8; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 8 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.

- Another aspect provides an isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of an isolated polynucleotide encoding a polypeptide of SEQ ID NO:74 or SEQ ID NO: 74 with at least one conservative amino acid substitution; SEQ ID NO: 73; an isolated polynucleotide that has at least 70%, 80%, 90%, or 95% sequence identity with SEQ ID NO: 73; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 73; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 73 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.

- Another aspect provides an isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3' where X is a hydrogen, Y is a hydrogen or a metal, R<sub>1</sub> and R<sub>2</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>3</sub> is selected from the group consisting of an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 74 or SEQ ID NO: 74 with at least one conservative amino acid substitution; SEQ ID NO: 73; an isolated polynucleotide that has at least 70%, 80%, 90%, or 95% sequence identity with SEQ ID NO: 73; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 73; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 73 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.

- Another aspect provides an isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 76 or SEQ ID NO: 76 with at least one conservative amino acid substitution; SEQ ID NO: 75; an isolated polynucleotide that has at least 70%, 80%, 90%, or 95% sequence identity with SEQ ID NO: 75; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 75; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 75 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.
- 10 Another aspect provides an isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3' where X is a hydrogen, Y is a hydrogen or a metal, R<sub>1</sub> and R<sub>2</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>2</sub> is selected from the group consisting of an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 76 or SEQ ID NO: 76 with at least one conservative amino acid substitution; SEQ ID NO: 75; an isolated polynucleotide that has at least 70%, 80%, 90%, or 95% sequence identity with SEQ ID NO: 75; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 75; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 75 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.
- 15 Another aspect provides an isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of SEQ ID NO: 42 or a degenerate variant thereof; an isolated polynucleotide that has at least 70%, 80%, 90%, or 95% sequence identity with SEQ ID NO: 42; an isolated polynucleotide of at least 10 amino acids that hybridizes under stringent conditions to SEQ ID NO: 42; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 42 and encodes an acyl CoA:cholesterol acyltransferase-like polypeptide.
- 20 Another aspect provides an isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3' where X is a hydrogen, Y is a hydrogen or a metal, R<sub>1</sub> and R<sub>2</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>2</sub> is selected from the group consisting of SEQ ID NO: 42 or a degenerate variant thereof; an isolated polynucleotide that has at least 70%, 80%, 90%, or 95% sequence identity with SEQ ID NO: 42; an isolated polynucleotide of at least 10 amino acids that hybridizes

under stringent conditions to SEQ ID NO: 42; an isolated polynucleotide complementary to any of the foregoing; and an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 42 and encodes a acyl CoA:cholesterol acyltransferase-like polypeptide.

5       Also provided is a recombinant nucleic acid construct comprising a regulatory sequence operably linked to a polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide and/or an acyl CoA:cholesterol acyltransferase-like polypeptide. In one embodiment, the sterol acyl transferases are plant sterol acyl transferases. In another embodiment, the recombinant nucleic acid constructs further 10 comprises a termination sequence. The regulatory sequence can be a constitutive promoter, an inducible promoter, a developmentally regulated promoter, a tissue specific promoter, an organelle specific promoter, a seed specific promoter or a combination of any of the foregoing. Also provided is a plant containing this recombinant nucleic acid construct and the seed and progeny from such a plant. This recombinant nucleic acid 15 construct can also be introduced into a suitable host cell to provide yet another aspect of the invention. If the host cell is a plant host cell, the cell can be used to generate a plant to provide another aspect of the invention. Further aspects include seed and progeny from such a plant.

20      Yet another aspect is a purified polypeptide comprising, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 74, SEQ ID NO: 76, or any of the preceding sequences with at least one conservative amino acid substitution.

25      Still another aspect provides a purified immunogenic polypeptide comprising at least 10 consecutive amino acids from an amino acid sequence selected from the group consisting of SEQ ID NO: 3, 5, 7, 9, 74, 76 and any of the preceding sequences containing at least one conservative amino acid substitution. Also provided are antibodies, either polyclonal or monoclonal, that specifically bind the preceding immunogenic polypeptides.

30      One aspect provides a method for producing a lecithin:cholesterol acyltransferase-like polypeptide or an acyl CoA:cholesterol acyltransferase-like polypeptide comprising culturing a host cell containing any recombinant nucleic acid construct of the present invention under condition permitting expression of said lecithin:cholesterol acyltransferase-like polypeptide or acyl CoA:cholesterol acyltransferase-like polypeptide.

Another aspect provides a method for modifying the sterol content of a host cell, comprising transforming a host cell with a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding a lecithin:cholesterol

acyltransferase-like polypeptide and culturing said host cell under conditions wherein said host cell expresses a lecithin:cholesterol acyltransferase-like polypeptide such that said host cell has a modified sterol composition as compared to host cells without the recombinant construct.

- 5 An additional aspect is a method for modifying the sterol content of a host cell comprising transforming a host cell with a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide and culturing said host cell under conditions wherein said host cell expresses an acyl CoA:cholesterol acyltransferase-like polypeptide such that said host cell has a modified sterol composition as compared to host cells without the recombinant construct.

- ) 10 A further aspect is a plant comprising a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide wherein expression of said recombinant construct results 15 in modified sterol composition of said plant as compared to the same plant without said recombinant construct.

- Another aspect provides a plant comprising a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide wherein expression of said recombinant construct results 20 in modified sterol composition of said plant as compared to the same plant without said recombinant construct.

In a further aspect is provided an oil obtained from any of the plants or host cells of the present invention.

- In still another aspect is provided a method for producing an oil with a modified 25 sterol composition comprising providing any of the plants or host cells of the present invention and extracting oil from the plant by any known method. Also provided is an oil produced by the preceding method.

- Still another aspect provides a method for altering oil production by a host cell comprising, transforming a host cell with a recombinant construct containing a regulatory 30 sequence operably linked to a polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide and culturing the host cell under conditions wherein the host cell expresses a lecithin:cholesterol acyltransferase-like polypeptide such that the host cell has an altered oil production as compared to host cells without the recombinant construct.

Another aspect provides a method for altering oil production by a host cell comprising, transforming a host cell with a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide and culturing the host cell under conditions wherein the host cell expresses an acyl CoA:cholesterol acyltransferase-like polypeptide such that the host cell has an altered oil production as compared to host cells without the recombinant construct.

Also provided is a plant comprising a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide wherein expression of said recombinant construct results in an altered production of oil by said plant as compared to the same plant without said recombinant construct.

In a further aspect is provided a plant comprising a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide wherein expression of said recombinant construct results in an altered production of oil by said plant as compared to the same plant without said recombinant construct.

Additional aspects provide a food, food ingredient or food product comprising any oil, plant or host cell of the present invention; a nutritional or dietary supplement comprising any oil, plant or host cell of the present invention; and a pharmaceutical composition comprising any oil, plant or host cell of the present invention along with a suitable diluent, carrier or excipient.

Additional aspects will be apparent from the descriptions and examples that follow.

25

## BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, appended claims and accompanying figures where:

Figure 1 shows an alignment of yeast, human and rat lecithin:cholesterol acyltransferase protein sequences with *Arabidopsis* LCAT1, LCAT2, LCAT3, and LCAT4 deduced amino acid sequences.

Figure 2 shows the results of NMR sterol ester analysis on T2 seed from plant expressing LCAT4 under the control of the napin promoter (pCGN9998).

Figure 3 shows the results of HPLC/MS sterol analysis on oil extracted from T2 seed from control lines (pCGN8640) and lines expressing LCAT3 (pCGN9968) under the control of the napin promoter.

Figure 4 shows the results of HPLC/MS sterol analysis on oil extracted from T2 seed from control lines (pCGN8640), and plant line expressing LCAT1 (pCGN9962), LCAT2 (pCGN9983), LCAT3 (pCGN9968), and LCAT4 (pCGN9998) under the control of the napin promoter. Additionally, data from 3 lines expressing LCAT4 under the control of the 35S promoter (pCGN9996) are shown.

Figure 5 shows the results of Nir analysis of the oil content of T2 seed from control lines (pCGN8640), and plant lines expressing LCAT1 (pCGN9962), LCAT2 (pCGN9983), and LCAT3 (pCGN9968) under the control of the napin promoter.

Additionally, data from 16 lines expressing LCAT2 under the control of the 35S promoter (pCGN9981) are shown.

15

#### DETAILED DESCRIPTION

The following detailed description is provided to aid those skilled in the art in practicing the present invention. Even so, this detailed description should not be construed to unduly limit the present invention as modifications and variations in the embodiments discussed herein can be made by those of ordinary skill in the art without departing from the spirit or scope of the present inventive discovery.

All publications, patents, patent applications and other references cited in this application are herein incorporated by reference in their entirety as if each individual publication, patent, patent application or other reference were specifically and individually indicated to be incorporated by reference.

The present invention relates to lecithin:cholesterol acyltransferase, particularly the isolated nucleic acid sequences encoding lecithin:cholesterol-like polypeptides (LCAT) from plant sources and acyl CoA:cholesterol:acyltransferase, particularly the isolated nucleic acid sequences encoding acyl CoA:cholesterol acyltransferase-like polypeptides (ACAT) from plant sources. Lecithin:cholesterol acyltransferase-like as used herein includes any nucleic acid sequence encoding an amino acid sequence from a plant source, such as a protein, polypeptide or peptide, obtainable from a cell source, which demonstrates the ability to utilize lecithin (phosphatidyl choline) as an acyl donor for acylation of sterols or glycerides to form esters under enzyme reactive conditions along with such proteins polypeptides and peptides. Acyl CoA:cholesterol acyltransferase-like

as used herein includes any nucleic acid sequence encoding an amino acid sequence from a plant source, such as a protein, polypeptide or peptide, obtainable from a cell source, which demonstrates the ability to utilize acyl CoA as an acyl donor for acylation of sterols or glycerides to form esters under enzyme reactive conditions along with such proteins 5 polypeptides and peptides. By "enzyme reactive conditions" is meant that any necessary conditions are available in an environment (i.e., such factors as temperature, pH, lack of inhibiting substances) which will permit the enzyme to function.

The term "sterol" as applied to plants refers to any chiral tetracyclic isopentenoid which may be formed by cyclization of squalene oxide through the transition state 10 possessing stereochemistry similar to the *trans-syn-trans-anti-trans-anti* configuration, for example, protosteroid cation, and which retains a polar group at C-3 (hydroxyl or keto), an *all-trans-anti* stereochemistry in the ring system, and a side-chain 20R-configuration (Parker, et al. (1992) In Nes, et al., Eds., *Regulation of Isopentenoid Metabolism*, ACS Symposium Series No. 497, p. 110; American Chemical Society, Washington, D.C.).

15 Sterols may or may not contain a C-5-C-6 double bond, as this is a feature introduced late in the biosynthetic pathway. Sterols contain a C<sub>8</sub>-C<sub>10</sub> side chain at the C-17 position.

The term "phytosterol," which applies to sterols found uniquely in plants, refers to a sterol containing a C-5, and in some cases a C-22, double bond. Phytosterols are further 20 characterized by alkylation of the C-17 side-chain with a methyl or ethyl substituent at the C-24 position. Major phytosterols include, but are not limited to, sitosterol, stigmasterol, campesterol, brassicasterol, etc. Cholesterol, which lacks a C-24 methyl or ethyl side-chain, is found in plants, but is not unique thereto, and is not a "phytosterol."

"Phytstanols" are saturated forms of phytosterols wherein the C-5 and, when 25 present, C-22 double bond(s) is (are) reduced, and include, but are not limited to, sitostanol, campestanol, and 22-dihydrobrassicastanol.

"Sterol esters" are further characterized by the presence of a fatty acid or phenolic acid moiety rather than a hydroxyl group at the C-3 position.

The term "sterol" includes sterols, phytosterols, phytosterol esters, phytstanols, 30 and phytostanol esters.

The term "sterol compounds" includes sterols, phytsterols, phytosterol esters, phytstanols, and phytostanol esters.

The term "phytosterol compound" refers to at least one phytosterol, at least one phytosterol ester, or a mixture thereof.

The term "phytostanol compound" refers to at least one phytostanol, at least one phytostanol ester, or a mixture thereof.

The term "glyceride" refers to a fatty acid ester of glycerol and includes mono-, di-, and tri- acylglycerols.

- 5 As used herein, "recombinant construct" is defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence, typically selection or production.
- 10 Alternatively, in terms of structure, it can be a sequence comprising fusion of two or more nucleic acid sequences which are not naturally contiguous or operatively linked to each other

) As used herein, "regulatory sequence" means a sequence of DNA concerned with controlling expression of a gene; e.g. promoters, operators and attenuators. A "heterologous regulatory sequence" is one which differs from the regulatory sequence naturally associated with a gene.

15 As used herein, "polynucleotide" and "oligonucleotide" are used interchangeably and mean a polymer of at least two nucleotides joined together by a phosphodiester bond and may consist of either ribonucleotides or deoxynucleotides.

20 As used herein, "sequence" means the linear order in which monomers appear in a polymer, for example, the order of amino acids in a polypeptide or the order of nucleotides in a polynucleotide.

) As used herein, "polypeptide", "peptide", and "protein" are used interchangeably and mean a compound that consist of two or more amino acids that are linked by means of peptide bonds.

25 As used herein, the terms "complementary" or "complementarity" refer to the pairing of bases, purines and pyrimidines, that associate through hydrogen bonding in double stranded nucleic acids. For example, the following base pairs are complementary: guanine and cytosine; adenine and thymine; and adenine and uracil. The terms, as used herein, include complete and partial complementarity.

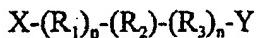
### Isolated proteins, Polypeptides and Polynucleotides

A first aspect of the present invention relates to isolated LCAT polynucleotides.

- The polynucleotide sequences of the present invention include isolated polynucleotides that encode the polypeptides of the invention having a deduced amino acid sequence  
5 selected from the group of sequences set forth in the Sequence Listing and to other polynucleotide sequences closely related to such sequences and variants thereof.

The invention provides a polynucleotide sequence identical over its entire length to each coding sequence as set forth in the Sequence Listing. The invention also provides the coding sequence for the mature polypeptide or a fragment thereof, as well as the coding  
10 sequence for the mature polypeptide or a fragment thereof in a reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, pro-, or prepro- protein sequence. The polynucleotide can also include non-coding sequences, including for example, but not limited to, non-coding 5' and 3' sequences, such as the transcribed, untranslated sequences, termination signals, ribosome binding sites, sequences  
15 that stabilize mRNA, introns, polyadenylation signals, and additional coding sequence that encodes additional amino acids. For example, a marker sequence can be included to facilitate the purification of the fused polypeptide. Polynucleotides of the present invention also include polynucleotides comprising a structural gene and the naturally associated sequences that control gene expression.

20 The invention also includes polynucleotides of the formula:



wherein, at the 5' end, X is hydrogen, and at the 3' end, Y is hydrogen or a metal, R<sub>1</sub> and R<sub>3</sub> are any nucleic acid residue, n is an integer between 0 and 3000, preferably between 1 and 1000 and R<sub>2</sub> is a nucleic acid sequence of the invention, particularly a nucleic acid  
25 sequence selected from the group set forth in the Sequence Listing and preferably SEQ ID NOs: 2, 4, 6, 8, 10-29, 33, 42-51, 73 and 75. In the formula, R<sub>2</sub> is oriented so that its 5' end residue is at the left, bound to R<sub>1</sub>, and its 3' end residue is at the right, bound to R<sub>3</sub>. Any stretch of nucleic acid residues denoted by either R group, where R is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer.

30 The invention also relates to variants of the polynucleotides described herein that encode for variants of the polypeptides of the invention. Variants that are fragments of the polynucleotides of the invention can be used to synthesize full-length polynucleotides of the invention. Preferred embodiments are polynucleotides encoding polypeptide variants wherein 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues of a polypeptide sequence of

the invention are substituted, added or deleted, in any combination. Particularly preferred are substitutions, additions, and deletions that are silent such that they do not alter the properties or activities of the polynucleotide or polypeptide.

Further preferred embodiments of the invention that are at least 50%, 60%, or 70% identical over their entire length to a polynucleotide encoding a polypeptide of the invention, and polynucleotides that are complementary to such polynucleotides. More preferable are polynucleotides that comprise a region that is at least 80% identical over its entire length to a polynucleotide encoding a polypeptide of the invention and polynucleotides that are complementary thereto. In this regard, polynucleotides at least 90% identical over their entire length are particularly preferred, those at least 95% identical are especially preferred. Further, those with at least 97% identity are highly preferred and those with at least 98% and 99% identity are particularly highly preferred, with those at least 99% being the most highly preferred.

Preferred embodiments are polynucleotides that encode polypeptides that retain substantially the same biological function or activity as determined by the methods described herein as the mature polypeptides encoded by the polynucleotides set forth in the Sequence Listing.

The invention further relates to polynucleotides that hybridize to the above-described sequences. In particular, the invention relates to polynucleotides that hybridize under stringent conditions to the above-described polynucleotides. An example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at approximately 65°C. Also included are polynucleotides that hybridize under a wash stringency of 0.1X SSC or 0.1X SSPE (at 50°C. Other hybridization and wash conditions are well known and are exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY (1989), particularly Chapter 11.

The invention also provides a polynucleotide consisting essentially of a polynucleotide sequence obtainable by screening an appropriate library containing the complete gene for a polynucleotide sequence set for in the Sequence Listing under stringent hybridization conditions with a probe having the sequence of said polynucleotide

sequence or a fragment thereof; and isolating said polynucleotide sequence. Methods for screening libraries are well known in the art and can be found for example in Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, NY (1989), particularly Chapter 8 and Ausubel et al., *Short Protocols in Molecular Biology*, 5 3<sup>rd</sup> ed, Wiley and Sons, 1995, chapter 6. Nucleic acid sequences useful for obtaining such a polynucleotide include, for example, probes and primers as described herein and in particular SEQ ID NO: 2, 4, 6, 8, 10-29, 33, 42-51, 73 and 75. These sequences are particularly useful in screening libraries obtained from *Arabidopsis*, soybean and corn for sequences encoding lecithin:cholesterol acyltransferase and lecithin:cholesterol acyltransferase-like polypeptides and for screening libraries for sequences encoding acyl CoA:cholesterol acyl transferase and acyl CoA:cholesterol acyl transferase-like polypeptides.

As discussed herein regarding polynucleotide assays of the invention, for example, polynucleotides of the invention can be used as a hybridization probe for RNA, cDNA, or genomic DNA to isolate full length cDNAs or genomic clones encoding a polypeptide and to isolate cDNA or genomic clones of other genes that have a high sequence similarity to a polynucleotide set forth in the Sequence Listing and in particular SEQ ID NO: 2, 4, 6, 8, 10-29, 33, 42-51, 73 and 75. Such probes will generally comprise at least 15 bases. Preferably such probes will have at least 30 bases and can have at least 50 bases. 20 Particularly preferred probes will have between 30 bases and 50 bases, inclusive.

The coding region of each gene that comprises or is comprised by a polynucleotide sequence set forth in the Sequence Listing may be isolated by screening using a DNA sequence provided in the Sequence Listing to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the 25 invention is then used to screen a library of cDNA, genomic DNA or mRNA to identify members of the library which hybridize to the probe. For example, synthetic oligonucleotides are prepared which correspond to the LCAT EST sequences. The oligonucleotides are used as primers in polymerase chain reaction (PCR) techniques to obtain 5' and 3' terminal sequence of LCAT genes. Alternatively, where oligonucleotides 30 of low degeneracy can be prepared from particular LCAT peptides, such probes may be used directly to screen gene libraries for LCAT gene sequences. In particular, screening of cDNA libraries in phage vectors is useful in such methods due to lower levels of background hybridization.

Typically, a LCAT sequence obtainable from the use of nucleic acid probes will show 60-70% sequence identity between the target LCAT sequence and the encoding sequence used as a probe. However, lengthy sequences with as little as 50-60% sequence identity may also be obtained. The nucleic acid probes may be a lengthy fragment of the 5 nucleic acid sequence, or may also be a shorter, oligonucleotide probe. When longer nucleic acid fragments are employed as probes (greater than about 100 bp), one may screen at lower stringencies in order to obtain sequences from the target sample which have 20-50% deviation (i.e., 50-80% sequence homology) from the sequences used as probe. Oligonucleotide probes can be considerably shorter than the entire nucleic acid 10 sequence encoding an LCAT enzyme, but should be at least about 10, preferably at least about 15, and more preferably at least about 20 nucleotides. A higher degree of sequence identity is desired when shorter regions are used as opposed to longer regions. It may thus be desirable to identify regions of highly conserved amino acid sequence to design oligonucleotide probes for detecting and recovering other related LCAT genes. Shorter 15 probes are often particularly useful for polymerase chain reactions (PCR), especially when highly conserved sequences can be identified. (See, Gould, et al., PNAS USA (1989) 86:1934-1938.).

Another aspect of the present invention relates to LCAT polypeptides. Such polypeptides include isolated polypeptides set forth in the Sequence Listing, as well as 20 polypeptides and fragments thereof, particularly those polypeptides which exhibit LCAT activity and also those polypeptides which have at least 50%, 60% or 70% identity, preferably at least 80% identity, more preferably at least 90% identity, and most preferably at least 95% identity to a polypeptide sequence selected from the group of sequences set forth in the Sequence Listing, and also include portions of such polypeptides, wherein 25 such portion of the polypeptide preferably includes at least 30 amino acids and more preferably includes at least 50 amino acids.

“Identity”, as is well understood in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, “identity” also means the degree of sequence 30 relatedness between polypeptide or polynucleotide sequences, as determined by the match between strings of such sequences. “Identity” can be readily calculated by known methods including, but not limited to, those described in *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York (1988); *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis*

- of Sequence Data, Part I*, Griffin, A.M. and Griffin, H.G., eds., Humana Press, New Jersey (1994); *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press (1987); *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., Stockton Press, New York (1991); and Carillo, H., and Lipman, D., *SIAM J Applied Math*, 48:1073 (1988).
- 5    Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available programs. Computer programs which can be used to determine identity between two sequences include, but are not limited to, GCG (Devereux, J., et al., *Nucleic Acids Research* 12(1):387 (1984); suite of five BLAST programs, three designed for nucleotide
- 10   sequences queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, *Trends in Biotechnology*, 12: 76-80 (1994); Birren, et al., *Genome Analysis*, 1: 543-559 (1997)). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH, Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.*, 215:403-410 (1990)). The well known Smith Waterman algorithm can also be used to determine
- 15   identity.

Parameters for polypeptide sequence comparison typically include the following:

Algorithm: Needleman and Wunsch, *J. Mol. Biol.* 48:443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl.*

20   *Acad. Sci USA* 89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

A program which can be used with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison Wisconsin. The above parameters along with no penalty for end gap are the default parameters for peptide comparisons.

Parameters for polynucleotide sequence comparison include the following:

Algorithm: Needleman and Wunsch, *J. Mol. Biol.* 48:443-453 (1970)

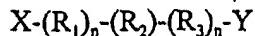
Comparison matrix: matches = +10; mismatches = 0

30   Gap Penalty: 50

Gap Length Penalty: 3

A program which can be used with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison Wisconsin. The above parameters are the default parameters for nucleic acid comparisons.

The invention also includes polypeptides of the formula:



- wherein, at the amino terminus, X is hydrogen, and at the carboxyl terminus, Y is hydrogen or a metal, R<sub>1</sub> and R<sub>3</sub> are any amino acid residue, n is an integer between 0 and 5 1000, and R<sub>2</sub> is an amino acid sequence of the invention, particularly an amino acid sequence selected from the group set forth in the Sequence Listing and preferably SEQ ID NOS: 3, 5, 7, 9, 74 and 76. In the formula, R<sub>2</sub> is oriented so that its amino terminal residue is at the left, bound to R<sub>1</sub>, and its carboxy terminal residue is at the right, bound to R<sub>3</sub>. Any stretch of amino acid residues denoted by either R group, where R is greater than 1, 10 may be either a heteropolymer or a homopolymer, preferably a heteropolymer.

) Polypeptides of the present invention include isolated polypeptides encoded by a polynucleotide comprising a sequence selected from the group of a sequence contained in SEQ ID NOS: 2, 4, 6, 8, 73 and 75.

- The polypeptides of the present invention can be mature protein or can be part of a 15 fusion protein.

Fragments and variants of the polypeptides are also considered to be a part of the invention. A fragment is a variant polypeptide which has an amino acid sequence that is entirely the same as part but not all of the amino acid sequence of the previously described 20 polypeptides. The fragments can be "free-standing" or comprised within a larger polypeptide of which the fragment forms a part or a region, most preferably as a single continuous region. Preferred fragments are biologically active fragments which are those fragments that mediate activities of the polypeptides of the invention, including those with similar activity or improved activity or with a decreased activity. Also included are those 25 polypeptides and polypeptide fragments that are antigenic or immunogenic in an animal, particularly a human and antibodies, either polyclonal or monoclonal that specifically bind the antigenic fragments. In one preferred embodiment, such antigenic or immunogenic fragments comprise at least 10 consecutive amino acids from the amino acid sequences disclosed herein or such sequences with at least one conservative amino acid substitution. In additional embodiments, such antigenic or immunogenic fragments comprise at least 30 15, at least 25, at least 50 or at least 100 consecutive amino acids from the amino acid sequences disclosed herein or such sequences with at least one conservative amino acid substitution. Methods for the production of antibodies from polypeptides and polypeptides conjugated to carrier molecules are well known in the art and can be found

for example in Ausubel et al., *Short Protocols in Molecular Biology*, 3<sup>rd</sup> ed., Wiley & Sons, 1995, particularly chapter 11.

Variants of the polypeptide also include polypeptides that vary from the sequences set forth in the Sequence Listing by conservative amino acid substitutions, substitution of 5 a residue by another with like characteristics. Those of ordinary skill in the art are aware that modifications in the amino acid sequence of a peptide, polypeptide, or protein can result in equivalent, or possibly improved, second generation peptides, etc., that display equivalent or superior functional characteristics when compared to the original amino-acid sequence. The present invention accordingly encompasses such modified amino acid 10 sequences. Alterations can include amino acid insertions, deletions, substitutions, truncations, fusions, shuffling of subunit sequences, and the like, provided that the peptide sequences produced by such modifications have substantially the same functional properties as the naturally occurring counterpart sequences disclosed herein.

One factor that can be considered in making such changes is the hydropathic index 15 of amino acids. The importance of the hydropathic amino acid index in conferring interactive biological function on a protein has been discussed by Kyte and Doolittle (*J. Mol. Biol.*, 157: 105-132, 1982). It is accepted that the relative hydropathic character of amino acids contributes to the secondary structure of the resultant protein. This, in turn, affects the interaction of the protein with molecules such as enzymes, substrates, receptors, 20 DNA, antibodies, antigens, etc.

Based on its hydrophobicity and charge characteristics, each amino acid has been assigned a hydropathic index as follows: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); 25 histidine (-3.2); glutamate/glutamine/aspartate/asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

As is known in the art, certain amino acids in a peptide or protein can be substituted for other amino acids having a similar hydropathic index or score and produce a resultant peptide or protein having similar biological activity, i.e., which still retains 30 biological functionality. In making such changes, it is preferable that amino acids having hydropathic indices within  $\pm 2$  are substituted for one another. More preferred substitutions are those wherein the amino acids have hydropathic indices within  $\pm 1$ . Most preferred substitutions are those wherein the amino acids have hydropathic indices within  $\pm 0.5$ .

Like amino acids can also be substituted on the basis of hydrophilicity. U.S. Patent No. 4,554,101 discloses that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. The following hydrophilicity values have been assigned to amino acids: arginine/lysine (+3.0); aspartate/glutamate (+3.0 ±1); serine (+0.3); asparagine/glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ±1); alanine/histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine/isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); and tryptophan (-3.4). Thus, one amino acid in a peptide, polypeptide, or protein can be substituted by another amino acid having a similar hydrophilicity score and still produce a resultant protein having similar biological activity, i.e., still retaining correct biological function. In making such changes, amino acids having hydropathic indices within ±2 are preferably substituted for one another, those within ±1 are more preferred, and those within ±0.5 are most preferred.

As outlined above, amino acid substitutions in the peptides of the present invention can be based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, etc. Exemplary substitutions that take various of the foregoing characteristics into consideration in order to produce conservative amino acid changes resulting in silent changes within the present peptides, etc., can be selected from other members of the class to which the naturally occurring amino acid belongs. Amino acids can be divided into the following four groups: (1) acidic amino acids; (2) basic amino acids; (3) neutral polar amino acids; and (4) neutral non-polar amino acids. Representative amino acids within these various groups include, but are not limited to: (1) acidic (negatively charged) amino acids such as aspartic acid and glutamic acid; (2) basic (positively charged) amino acids such as arginine, histidine, and lysine; (3) neutral polar amino acids such as glycine, serine, threonine, cysteine, cystine, tyrosine, asparagine, and glutamine; and (4) neutral non-polar amino acids such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine. It should be noted that changes which are not expected to be advantageous can also be useful if these result in the production of functional sequences.

Variants that are fragments of the polypeptides of the invention can be used to produce the corresponding full length polypeptide by peptide synthesis. Therefore, these variants can be used as intermediates for producing the full-length polypeptides of the invention.

The polynucleotides and polypeptides of the invention can be used, for example, in the transformation of host cells, such as plant cells, animal cells, yeast cells, bacteria, bacteriophage, and viruses, as further discussed herein.

- The invention also provides polynucleotides that encode a polypeptide that is a mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids within the mature polypeptide (for example, when the mature form of the protein has more than one polypeptide chain). Such sequences can, for example, play a role in the processing of a protein from a precursor to a mature form, allow protein transport, shorten or lengthen protein half-life, or facilitate manipulation of the protein in assays or production. It is contemplated that cellular enzymes can be used to remove any additional amino acids from the mature protein.

- A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. The inactive precursors generally are activated when the prosequences are removed. Some or all of the prosequences may be removed prior to activation. Such precursor protein are generally called proproteins.

#### Preparation of Expression Constructs and Methods of Use

- Of interest is the use of the nucleotide sequences in recombinant DNA constructs to direct the transcription or transcription and translation (expression) of the acyltransferase sequences of the present invention in a host cell. Of particular interest is the use of the polynucleotide sequences of the present invention in recombinant DNA constructs to direct the transcription or transcription and translation (expression) of the acyltransferase sequences of the present invention in a host plant cell.
- The expression constructs generally comprise a regulatory sequence functional in a host cell operably linked to a nucleic acid sequence encoding a lecithin:cholesterol acyltransferase-like polypeptide or acyl CoA:cholesterol acyltransferase-like polypeptide of the present invention and a transcriptional termination region functional in a host plant cell. Of particular interest is the use of promoters (also referred to as transcriptional initiation regions) functional in plant host cells.

Those skilled in the art will recognize that there are a number of promoters which are functional in plant cells, and have been described in the literature including constitutive, inducible, tissue specific, organelle specific, developmentally regulated and environmentally regulated promoters. Chloroplast and plastid specific promoters,

chloroplast or plastid functional promoters, and chloroplast or plastid operable promoters are also envisioned.

- One set of promoters are constitutive promoters such as the CaMV35S or FMV35S promoters that yield high levels of expression in most plant organs. Enhanced or 5 duplicated versions of the CaMV35S and FMV35S promoters are useful in the practice of this invention (Odell, et al. (1985) *Nature* 313:810-812; Rogers, U.S. Patent Number 5,378,619). Other useful constitutive promoters include, but are not limited to, the mannopine synthase (*mas*) promoter, the nopaline synthase (*nos*) promoter, and the octopine synthase (*ocs*) promoter.
- 10 Useful inducible promoters include heat-shock promoters (Ou-Lee et al. (1986) *Proc. Natl. Acad. Sci. USA* 83: 6815; Ainley et al. (1990) *Plant Mol. Biol.* 14: 949), a nitrate-inducible promoter derived from the spinach nitrite reductase gene (Back et al. (1991) *Plant Mol. Biol.* 17: 9), hormone-inducible promoters (Yamaguchi-Shinozaki et al. (1990) *Plant Mol. Biol.* 15: 905; Kares et al. (1990) *Plant Mol. Biol.* 15: 905), and 15 light-inducible promoters associated with the small subunit of RuBP carboxylase and LHCP gene families (Kuhlemeier et al. (1989) *Plant Cell* 1: 471; Feinbaum et al. (1991) *Mol. Gen. Genet.* 226: 449; Weisshaar et al. (1991) *EMBO J.* 10: 1777; Lam and Chua (1990) *Science* 248: 471; Castresana et al. (1988) *EMBO J.* 7: 1929; Schulze-Lefert et al. (1989) *EMBO J.* 8: 651).
- 20 In addition, it may also be preferred to bring about expression of the acyltransferase gene in specific tissues of the plant, such as leaf, stem, root, tuber, seed, fruit, etc., and the promoter chosen should have the desired tissue and developmental specificity. Examples of useful tissue-specific, developmentally-regulated promoters include fruit-specific promoters such as the E4 promoter (Cordes et al. (1989) *Plant Cell* 1:1025), the E8 promoter (Deikman et al. (1988) *EMBO J.* 7: 3315), the kiwifruit actinidin promoter (Lin et al. (1993) *PNAS* 90: 5939), the 2A11 promoter (Houck et al., U.S. Patent 4,943,674), and the tomato pZ130 promoter (U.S. Patents 5,175,095 and 5,530,185); the  $\beta$ -conglycinin 7S promoter (Doyle et al. (1986) *J. Biol. Chem.* 261: 9228; Slighton and Beachy (1987) *Planta* 172: 356), and seed-specific promoters (Knutzon et al. (1992) *Proc. Natl. Acad. Sci. USA* 89: 2624; Bustos et al. (1991) *EMBO J.* 10: 1469; Lam and Chua (1991) *J. Biol. Chem.* 266: 17131; Stayton et al. (1991) *Aust. J. Plant. Physiol.* 18: 507). Fruit-specific gene regulation is discussed in U.S. Patent 5,753,475. Other 25 useful seed-specific promoters include, but are not limited to, the napin, phaseolin, zein, soybean trypsin inhibitor, 7S, ADR12, ACP, stearoyl-ACP desaturase, oleosin,

*Lasquerella* hydroxylase, and barley aldose reductase promoters (Bartels (1995) *Plant J.* 7: 809-822), the EA9 promoter (U.S. Patent 5,420,034), and the Bce4 promoter (U.S. Patent 5,530,194). Useful embryo-specific promoters include the corn globulin 1 and oleosin promoters. Useful endosperm-specific promoters include the rice glutelin-1 promoter, the 5 promoters for the low-pI  $\beta$  amylase gene (Amy32b) (Rogers et al. (1984) *J. Biol. Chem.* 259: 12234), the high-pI  $\beta$  amylase gene (Amy 64) (Khurseed et al. (1988) *J. Biol. Chem.* 263: 18953), and the promoter for a barley thiol protease gene ("Aleurain") (Whittier et al. (1987) *Nucleic Acids Res.* 15: 2515).

Of particular interest is the expression of the nucleic acid sequences of the present 10 invention from transcription initiation regions which are preferentially expressed in a plant seed tissue. Examples of such seed preferential transcription initiation sequences include those sequences derived from sequences encoding plant storage protein genes or from genes involved in fatty acid biosynthesis in oilseeds. Examples of such promoters include the 5' regulatory regions from such genes as napin (Kridl et al., *Seed Sci. Res.* 1:209:219 15 (1991)), phaseolin, zein, soybean trypsin inhibitor, ACP, stearoyl-ACP desaturase, soybean  $\alpha'$  subunit of  $\beta$ -conglycinin (soy 7s, (Chen et al., *Proc. Natl. Acad. Sci.*, 83:8560-8564 (1986))) and oleosin. Seed-specific gene regulation is discussed in EP 0 255 378 B1 and U.S. Patents 5,420,034 and 5,608,152. Promoter hybrids can also be constructed to enhance transcriptional activity (Hoffman, U.S. Patent No. 5,106,739), or to combine 20 desired transcriptional activity and tissue specificity.

It may be advantageous to direct the localization of proteins conferring LCAT to a particular subcellular compartment, for example, to the mitochondrion, endoplasmic reticulum, vacuoles, chloroplast or other plastidic compartment. For example, where the genes of interest of the present invention will be targeted to plastids, such as chloroplasts, 25 for expression, the constructs will also employ the use of sequences to direct the gene to the plastid. Such sequences are referred to herein as chloroplast transit peptides (CTP) or plastid transit peptides (PTP). In this manner, where the gene of interest is not directly inserted into the plastid, the expression construct will additionally contain a gene encoding a transit peptide to direct the gene of interest to the plastid. The chloroplast transit 30 peptides may be derived from the gene of interest, or may be derived from a heterologous sequence having a CTP. Such transit peptides are known in the art. See, for example, Von Heijne et al. (1991) *Plant Mol. Biol. Rep.* 9:104-126; Clark et al. (1989) *J. Biol. Chem.* 264:17544-17550; della-Cioppa et al. (1987) *Plant Physiol.* 84:965-968; Romer et al.

(1993) *Biochem. Biophys. Res Commun.* 196:1414-1421; and, Shah *et al.* (1986) *Science* 233:478-481.

Depending upon the intended use, the constructs may contain the nucleic acid sequence which encodes the entire LCAT protein, a portion of the LCAT protein, the 5 entire ACAT protein, or a portion of the ACAT protein. For example, where antisense inhibition of a given LCAT or ACAT protein is desired, the entire sequence is not required. Furthermore, where LCAT or ACAT sequences used in constructs are intended for use as probes, it may be advantageous to prepare constructs containing only a particular portion of a LCAT or ACAT encoding sequence, for example a sequence which 10 is discovered to encode a highly conserved region.

The skilled artisan will recognize that there are various methods for the inhibition of expression of endogenous sequences in a host cell. Such methods include, but are not limited to antisense suppression (Smith, *et al.* (1988) *Nature* 334:724-726), co-suppression (Napoli, *et al.* (1989) *Plant Cell* 2:279-289), ribozymes (PCT Publication 15 WO 97/10328), and combinations of sense and antisense Waterhouse, *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 95:13959-13964. Methods for the suppression of endogenous sequences in a host cell typically employ the transcription or transcription and translation of at least a portion of the sequence to be suppressed. Such sequences may be homologous to coding as well as non-coding regions of the endogenous sequence.

Regulatory transcript termination regions may be provided in plant expression 20 constructs of this invention as well. Transcript termination regions may be provided by the DNA sequence encoding the diacylglycerol acyltransferase or a convenient transcription termination region derived from a different gene source, for example, the transcript termination region which is naturally associated with the transcript initiation 25 region. The skilled artisan will recognize that any convenient transcript termination region which is capable of terminating transcription in a plant cell may be employed in the constructs of the present invention.

Alternatively, constructs may be prepared to direct the expression of the LCAT or ACAT sequences directly from the host plant cell plastid. Such constructs and methods 30 are known in the art and are generally described, for example, in Svab, *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:8526-8530 and Svab and Maliga (1993) *Proc. Natl. Acad. Sci. USA* 90:913-917 and in U.S. Patent Number 5,693,507.

A plant cell, tissue, organ, or plant into which the recombinant DNA constructs containing the expression constructs have been introduced is considered transformed,

transfected, or transgenic. A transgenic or transformed cell or plant also includes progeny of the cell or plant and progeny produced from a breeding program employing such a transgenic plant as a parent in a cross and exhibiting an altered phenotype resulting from the presence of a LCAT nucleic acid sequence.

5       Plant expression or transcription constructs having a plant LCAT as the DNA sequence of interest for increased or decreased expression thereof may be employed with a wide variety of plant life, particularly, plant life involved in the production of vegetable oils for edible and industrial uses. Most especially preferred are temperate oilseed crops. Plants of interest include, but are not limited to, rapeseed (Canola and High Erucic Acid 10 varieties), sunflower, safflower, cotton, soybean, peanut, coconut and oil palms, and corn. Depending on the method for introducing the recombinant constructs into the host cell, other DNA sequences may be required. Importantly, this invention is applicable to dicotyledons and monocotyledons species alike and will be readily applicable to new and/or improved transformation and regulation techniques.

15      Of particular interest, is the use of plant LCAT and ACAT constructs in plants to produce plants or plant parts, including, but not limited to leaves, stems, roots, reproductive, and seed, with a modified content of lipid and/or sterol esters and to alter the oil production by such plants.

20      Of particular interest in the present invention, is the use of ACAT genes in conjunction with the LCAT sequences to increase the sterol content of seeds. Thus, overexpression of a nucleic acid sequence encoding an ACAT and LCAT in an oilseed crop may find use in the present invention to increase sterol levels in plant tissues and/or increase oil production.

25      It is contemplated that the gene sequences may be synthesized, either completely or in part, especially where it is desirable to provide plant-preferred sequences. Thus, all or a portion of the desired structural gene (that portion of the gene which encodes the LCAT or ACAT protein) may be synthesized using codons preferred by a selected host. Host-preferred codons may be determined, for example, from the codons used most frequently in the proteins expressed in a desired host species.

30      One skilled in the art will readily recognize that antibody preparations, nucleic acid probes (DNA and RNA) and the like may be prepared and used to screen and recover "homologous" or "related" sequences from a variety of plant sources. Homologous sequences are found when there is an identity of sequence, which may be determined upon comparison of sequence information, nucleic acid or amino acid, or through hybridization

reactions between a known LCAT and a candidate source. Conservative changes, such as Glu/Asp, Val/Ile, Ser/Thr, Arg/Lys and Gln/Asn may also be considered in determining sequence homology. Amino acid sequences are considered homologous by as little as 25% sequence identity between the two complete mature proteins. (See generally,

- 5 Doolittle, R.F., *OF URFS and ORFS* (University Science Books, CA, 1986.)

Thus, other LCATs may be obtained from the specific sequences provided herein. Furthermore, it will be apparent that one can obtain natural and synthetic sequences, including modified amino acid sequences and starting materials for synthetic-protein modeling from the exemplified LCAT and ACAT sequences and from sequences which 10 are obtained through the use of such exemplified sequences. Modified amino acid sequences include sequences which have been mutated, truncated, increased and the like, whether such sequences were partially or wholly synthesized. Sequences which are actually purified from plant preparations or are identical or encode identical proteins thereto, regardless of the method used to obtain the protein or sequence, are equally 15 considered naturally derived.

For immunological screening, antibodies to the protein can be prepared by injecting rabbits or mice with the purified protein or portion thereof, such methods of preparing antibodies being well known to those in the art. Either monoclonal or polyclonal antibodies can be produced, although typically polyclonal antibodies are more 20 useful for gene isolation. Western analysis may be conducted to determine that a related protein is present in a crude extract of the desired plant species, as determined by cross-reaction with the antibodies to the encoded proteins. When cross-reactivity is observed, genes encoding the related proteins are isolated by screening expression libraries representing the desired plant species. Expression libraries can be constructed in a variety 25 of commercially available vectors, including lambda gt11, as described in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

To confirm the activity and specificity of the proteins encoded by the identified nucleic acid sequences as acyltransferase enzymes, *in vitro* assays are performed in insect 30 cell cultures using baculovirus expression systems. Such baculovirus expression systems are known in the art and are described by Lee, *et al.* U.S. Patent Number 5,348,886, the entirety of which is herein incorporated by reference.

In addition, other expression constructs may be prepared to assay for protein activity utilizing different expression systems. Such expression constructs are transformed

into yeast or prokaryotic host and assayed for acyltransferase activity. Such expression systems are known in the art and are readily available through commercial sources.

- The method of transformation in obtaining such transgenic plants is not critical to the instant invention, and various methods of plant transformation are currently available.
- 5 Furthermore, as newer methods become available to transform crops, they may also be directly applied hereunder. For example, many plant species naturally susceptible to *Agrobacterium* infection may be successfully transformed via tripartite or binary vector methods of *Agrobacterium* mediated transformation. In many instances, it will be desirable to have the construct bordered on one or both sides by T-DNA, particularly
- 10 having the left and right borders, more particularly the right border. This is particularly useful when the construct uses *A. tumefaciens* or *A. rhizogenes* as a mode for transformation, although the T-DNA borders may find use with other modes of transformation. In addition, techniques of microinjection, DNA particle bombardment, and electroporation have been developed which allow for the transformation of various
- 15 monocot and dicot plant species.

- Normally, included with the DNA construct will be a structural gene having the necessary regulatory regions for expression in a host and providing for selection of transformant cells. The gene may provide for resistance to a cytotoxic agent, e.g. antibiotic, heavy metal, toxin, etc., complementation providing prototrophy to an
- 20 auxotrophic host, viral immunity or the like. Depending upon the number of different host species the expression construct or components thereof are introduced, one or more markers may be employed, where different conditions for selection are used for the different hosts.

- Non-limiting examples of suitable selection markers include genes that confer
- 25 resistance to bleomycin, gentamycin, glyphosate, hygromycin, kanamycin, methotrexate, phleomycin, phosphinotricin, spectinomycin, streptomycin, sulfonamide and sulfonylureas. Maliga et al., *Methods in Plant Molecular Biology*, Cold Spring Harbor Laboratory Press, 1995, p. 39. Examples of markers include, but are not limited to, alkaline phosphatase (AP), myc, hemagglutinin (HA),  $\beta$  glucuronidase (GUS), luciferase,
- 30 and green fluorescent protein (GFP).

Where *Agrobacterium* is used for plant cell transformation, a vector may be used which may be introduced into the *Agrobacterium* host for homologous recombination with T-DNA or the Ti- or Ri-plasmid present in the *Agrobacterium* host. The Ti- or Ri-plasmid containing the T-DNA for recombination may be armed (capable of causing gall

formation) or disarmed (incapable of causing gall formation), the latter being permissible, so long as the *vir* genes are present in the transformed *Agrobacterium* host. The armed plasmid can give a mixture of normal plant cells and gall.

In some instances where *Agrobacterium* is used as the vehicle for transforming 5 host plant cells, the expression or transcription construct bordered by the T-DNA border region(s) will be inserted into a broad host range vector capable of replication in *E. coli* and *Agrobacterium*, there being broad host range vectors described in the literature.

Commonly used is pRK2 or derivatives thereof. See, for example, Ditta, *et al.*, (*Proc. Nat. Acad. Sci., U.S.A.* (1980) 77:7347-7351) and EPA 0 120 515, which are incorporated 10 herein by reference. Alternatively, one may insert the sequences to be expressed in plant cells into a vector containing separate replication sequences, one of which stabilizes the vector in *E. coli*, and the other in *Agrobacterium*. See, for example, McBride and Summerfelt (*Plant Mol. Biol.* (1990) 14:269-276), wherein the pRiHRI (Jouanin, *et al.*, *Mol. Gen. Genet.* (1985) 201:370-374) origin of replication is utilized and provides for 15 added stability of the plant expression vectors in host *Agrobacterium* cells.

Included with the expression construct and the T-DNA can be one or more markers, which allow for selection of transformed *Agrobacterium* and transformed plant cells. A

number of markers have been developed for use with plant cells, such as resistance to 20 chloramphenicol, kanamycin, the aminoglycoside G418, hygromycin, or the like. The particular marker employed is not essential to this invention, one or another marker being preferred depending on the particular host and the manner of construction.

For transformation of plant cells using *Agrobacterium*, explants may be combined and incubated with the transformed *Agrobacterium* for sufficient time for transformation, 25 the bacteria killed, and the plant cells cultured in an appropriate selective medium. Once callus forms, shoot formation can be encouraged by employing the appropriate plant hormones in accordance with known methods and the shoots transferred to rooting medium for regeneration of plants. The plants may then be grown to seed and the seed used to establish repetitive generations and for isolation of vegetable oils.

Thus, in another aspect of the present invention, methods for modifying the sterol 30 and/or stanol composition of a host cell. Of particular interest are methods for modifying the sterol and/or stanol composition of a host plant cell. In general the methods involve either increasing the levels of sterol ester compounds as a proportion of the total sterol

compounds. The method generally comprises the use of expression constructs to direct the expression of the polynucleotides of the present invention in a host cell.

- Also provided are methods for reducing the proportion of sterol ester compounds as a percentage of total sterol compounds in a host plant cell. The method generally
- 5 comprises the use of expression constructs to direct the suppression of endogenous acyltransferase proteins in a host cell.

Of particular interest is the use of expression constructs to modify the levels of sterol compounds in a host plant cell. Most particular, the methods find use in modifying the levels of sterol compounds in seed oils obtained from plant seeds.

- 10 Also of interest is the use of expression constructs of the present invention to alter oil production in a host cell and in particular to increase oil production. Of particular interest is the use of expression constructs containing nucleic acid sequences encoding LCAT and/or ACAT polypeptides to transform host plant cells and to use these host cells to regenerate whole plants having increased oil production as compared to the same plant
- 15 not containing the expression construct.

- The oils obtained from transgenic plants having modified sterol compound content find use in a wide variety of applications. Of particular interest in the present invention is the use of the oils containing modified levels of sterol compounds in applications involved in improving human nutrition and cardiovascular health. For example, phytostanols are
- 20 beneficial for lowering serum cholesterol (Ling, *et al.* (1995) *Life Sciences* 57:195-206).

- Cholesterol-lowering compositions comprise the oils and sterol ester compound compositions obtained using the methods of the present invention. Such cholesterol lowering compositions include, but are not limited to foods, food products, processed foods, food ingredients, food additive compositions, or dietary/nutritional supplements
- 25 that contain oils and/or fats. Non-limiting examples include margarines; butters; shortenings; cooking oils; frying oils; dressings, such as salad dressings; spreads; mayonnaises; and vitamin/mineral supplements. Patent documents relating to such compositions include, U.S. Patents 4,588,717 and 5,244,887, and PCT International Publication Nos. WO 96/38047, WO 97/42830, WO 98/06405, and WO 98/06714.
- 30 Additional non-limiting examples include toppings; dairy products such as cheese and processed cheese; processed meat; pastas; sauces; cereals; desserts, including frozen and shelf-stable desserts; dips; chips; baked goods; pastries; cookies; snack bars; confections; chocolates; beverages; unextracted seed; and unextracted seed that has been

ground, cracked, milled, rolled, extruded, pelleted, defatted, dehydrated, or otherwise processed, but which still contains the oils, etc., disclosed herein.

The cholesterol-lowering compositions can also take the form of pharmaceutical compositions comprising a cholesterol-lowering effective amount of the oils or sterol compound compositions obtained using the methods of the present invention, along with a pharmaceutically acceptable carrier, excipient, or diluent. These pharmaceutical compositions can be in the form of a liquid or a solid. Liquids can be solutions or suspensions; solids can be in the form of a powder, a granule, a pill, a tablet, a gel, or an extrudate. U.S. Patent 5,270,041 relates to sterol-containing pharmaceutical compositions.

Thus, by expression of the nucleic acid sequences encoding acyltransferase-like sequences of the present invention in a host cell, it is possible to modify the lipid content and/or composition as well as the sterol content and/or composition of the host cell.

The invention now being generally described, it will be more readily understood by reference to the following examples which are included for purposes of illustration only and are not intended to limit the present invention.

## EXAMPLES

### Example 1: RNA Isolations

Total RNA from the inflorescence and developing seeds of *Arabidopsis thaliana* was isolated for use in construction of complementary (cDNA) libraries. The procedure was an adaptation of the DNA isolation protocol of Webb and Knapp (D.M. Webb and S.J. Knapp, (1990) Plant Molec. Reporter, 8, 180-185). The following description assumes the use of 1g fresh weight of tissue. Frozen seed tissue was powdered by grinding under liquid nitrogen. The powder was added to 10ml REC buffer (50mM Tris-HCl, pH 9, 0.8M NaCl, 10mM EDTA, 0.5% w/v CTAB (cetyltrimethyl-ammonium bromide)) along with 0.2g insoluble polyvinylpolypyrrolidone, and ground at room temperature. The homogenate was centrifuged for 5 minutes at 12,000 xg to pellet insoluble material. The resulting supernatant fraction was extracted with chloroform, and the top phase was recovered.

The RNA was then precipitated by addition of 1 volume RecP (50mM Tris-HCL pH9, 10mM EDTA and 0.5% (w/v) CTAB) and collected by brief centrifugation as before. The RNA pellet was redissolved in 0.4 ml of 1M NaCl. The RNA pellet was redissolved in water and extracted with phenol/chloroform. Sufficient 3M potassium acetate (pH 5) was added to make the mixture 0.3M in acetate, followed by addition of two volumes of

ethanol to precipitate the RNA. After washing with ethanol, this final RNA precipitate was dissolved in water and stored frozen.

Alternatively, total RNA may be obtained using TRIzol reagent (BRL-Lifetechnologies, Gaithersburg, MD) following the manufacturer's protocol. The RNA precipitate was dissolved in water and stored frozen.  
5

**Example 2: Identification of LCAT Sequences**

Searches were performed on a Silicon Graphics Unix computer using additional Bioaccelerator hardware and GenWeb software supplied by Compugen Ltd. This  
10 software and hardware enabled the use of the Smith-Waterman algorithm in searching DNA and protein databases using profiles as queries. The program used to query protein databases was profilesearch. This is a search where the query is not a single sequence but a profile based on a multiple alignment of amino acid or nucleic acid sequences. The profile was used to query a sequence data set, i.e., a sequence database. The profile  
15 contained all the pertinent information for scoring each position in a sequence, in effect replacing the "scoring matrix" used for the standard query searches. The program used to query nucleotide databases with a protein profile was tprofilesearch. Tprofilesearch searches nucleic acid databases using an amino acid profile query. As the search is running, sequences in the database are translated to amino acid sequences in six reading  
20 frames. The output file for tprofilesearch is identical to the output file for profilesearch except for an additional column that indicates the frame in which the best alignment occurred.

The Smith-Waterman algorithm, (Smith and Waterman (1981) *J. Molec. Biol.* 147:195-197), was used to search for similarities between one sequence from the query  
25 and a group of sequences contained in the database.

A protein sequence of Lecithin: cholesterol acyltransferase from human (McLean J, et al. (1986) *Nucleic Acids Res.* 14(23):9397-406 SEQ ID NO:1) was used to search the NCBI non-redundant protein database using BLAST. Three sequences were identified from *Arabidopsis*, GenBank accessions AC004557 (referred to herein as LCAT1, SEQ ID NO:2), AC003027 (referred to herein as LCAT2, SEQ ID NO:4), and AL024486 (referred to herein as LCAT3, SEQ ID NO:6). The deduced amino acid sequences are provided in SEQ ID NOS: 3, 5, and 7, respectively.  
30

The profile generated from the queries using PSI-BLAST was excised from the hyper text markup language (html) file. The worldwide web (www)/html interface to

psiblast at ncbi stores the current generated profile matrix in a hidden field in the html file that is returned after each iteration of psiblast. However, this matrix has been encoded into string62 (s62) format for ease of transport through html. String62 format is a simple conversion of the values of the matrix into html legal ascii characters.

5       The encoded matrix width (x axis) is 26 characters, and comprise the consensus characters, the probabilities of each amino acid in the order A,B,C,D,E,F,G,H,I,K,L,M,N, P,Q,R,S,T,V,W,X,Y,Z (where B represents D and N, and Z represents Q and E, and X represents any amino acid), gap creation value, and gap extension value.

10      The length (y axis) of the matrix corresponds to the length of the sequences identified by PSI-BLAST. The order of the amino acids corresponds to the conserved amino acid sequence of the sequences identified using PSI-BLAST, with the N-terminal end at the top of the matrix. The probabilities of other amino acids at that position are represented for each amino acid along the x axis, below the respective single letter amino acid abbreviation.

15      Thus, each row of the profile consists of the highest scoring (consensus) amino acid, followed by the scores for each possible amino acid at that position in sequence matrix, the score for opening a gap at that position, and the score for continuing a gap at that position.

20      The string62 file is converted back into a profile for use in subsequent searches. The gap open field is set to 11 and the gap extension field is set to 1 along the x axis. The gap creation and gap extension values are known, based on the settings given to the PSI-BLAST algorithm. The matrix is exported to the standard GCG profile form. This format can be read by GenWeb.

25      The algorithm used to convert the string62 formatted file to the matrix is outlined in Table 1.

Table 1

- |    |  |
|----|--|
|    | 1. if encoded character z then the value is blast score min                                  |
|    | 2. if encoded character Z then the value is blast score max                                  |
| 5  | 3. else if the encoded character is uppercase then its value is (64-(ascii # of char))       |
|    | 4. else if the encoded character is a digit the value is ((ascii # of char)-48)              |
|    | 5. else if the encoded character is not uppercase then the value is ((ascii # of char) - 87) |
|    | 6. ALL B positions are set to min of D and N amino acids at that row in sequence matrix      |
|    | 7. ALL Z positions are set to min of Q and E amino acids at that row in sequence matrix      |
| 10 | 8. ALL X positions are set to min of all amino acids at that row in sequence matrix          |
|    | 9. kBLAST_SCORE_MAX=999;   |
|    | 10. kBLAST_SCORE_MIN=-999;   |
|    | 11. all gap opens are set to 11  |
|    | 12. all gap lens are set to 1  |

15

The protein sequences of LCAT1, LCAT2, and LCAT3 as well as the PSI-BLAST profile were used to search public and proprietary databases for additional LCAT sequences. Two EST sequences were identified which appear to be identical to LCAT1 and LCAT3, respectively. One additional *Arabidopsis* sequence was identified from the proprietary databases, LCAT4 (SEQ ID NO:8). The deduced protein sequence of LCAT4 is provided in SEQ ID NO:9. Two additional genomic sequences were identified using the PSI-BLAST profile from libraries of *Arabidopsis* ecotypes Columbia and Landsberg, LCAT7 (SEQ ID NO:10) and LCAT8 (SEQ ID NO:11). The LCAT7 sequence was present in both the Columbia and Landsberg genomic libraries, while the LCAT8 sequence was only present in the Columbia library.

An open reading frame was predicted from the genomic sequence of LCAT7 in the *Arabidopsis* public database and this sequence was called MSH12 (referred to herein as LCAT5, SEQ ID NO: 73). The deduced protein sequence of LCAT5 is provided in SEQ ID NO: 74.

30 The PSI-BLAST profile and the LCAT sequences were used to query the public yeast database and proprietary libraries containing corn and soy EST sequences. The yeast genome contains only one gene, *LRO1* (LCAT Related Open reading frame, YNR008W, Figure 1) with distinct similarity to the human LCAT. The DNA sequence of *LRO1* is

provided in SEQ ID NO: 75 and the protein sequence is provided in SEQ ID NO: 76. Seven EST sequences were identified from soybean libraries as being LCAT sequences. Two sequences from soy (SEQ ID NOs: 12 and 13) are most closely related to the *Arabidopsis* LCAT1 sequence, a single sequence was identified as being most closely related to LCAT2 (SEQ ID NO:14), three were closely related to LCAT3 (SEQ ID NOs: 15-17), and an additional single sequence was identified (SEQ ID NO:18). A total of 11 corn EST sequences were identified as being related to the *Arabidopsis* LCAT sequences. Two corn EST sequences (SEQ ID NOs: 19 and 20) were most closely related to LCAT1, two sequences were identified as closely related to LCAT2 (SEQ ID NOs: 21 and 22), four corn EST sequences were identified as closely related to LCAT3 (SEQ ID NOs: 23-26), and an additional three corn EST sequences were also identified (SEQ ID NOs: 27-29).

### Example 3: Identification of ACAT Sequences

Since plant ACATs are unknown in the art, searches were performed to identify known and related ACAT sequences from mammalian sources from public databases. These sequences were then used to search public and proprietary EST databases to identify plant ACAT-like sequences.

A public database containing mouse Expressed Sequence Tag (EST) sequences (dBEST) was searched for ACAT-like sequences. The search identified two sequences (SEQ ID 30 and 31) which were related (approximately 20% identical), but divergent, to known ACAT sequences.

In order to identify ACAT-like sequences from other organisms, the two mouse ACAT sequences were used to search public and proprietary databases containing EST sequences from human and rat tissues. Results of the search identified several sequences from the human database and from the rat database which were closely related to the mouse sequences. The human and rat ACAT-like EST sequences were assembled, using the GCG assembly program, to construct a complete inferred cDNA sequence by identifying overlapping sequences (SEQ ID NOs: 32 and 33, respectively).

The protein sequence of the human ACAT-like sequence was aligned with known ACAT sequences from human (Chang, *et al.* (1993) *J. Biol. Chem.* 268:20747-20755, SEQ ID NO:34), mouse (Uelmen, *et al.* (1995) *J. Biol. Chem.* 270:26192-26201 SEQ ID NO:35) and yeast (Yu, *et al.* (1996) *J. Biol. Chem.* 271:24157-24163, SEQ ID NO:36 and Yang, *et al.* (1996) *Science* 272:1353-1356, SEQ ID NO:37) using MacVector (Oxford Molecular, Inc.). Results of the alignment demonstrated that the sequence was related to

the known sequences, however the related sequence was only about 25% similar to the known sequences.

The protein sequence of the human sterol O-acyltransferase (ACAT, Acyl CoA:Cholesterol acyltransferase, Accession number A48026) related sequence was used 5 to search protein and nucleic acid Genbank databases. A single plant homologue was identified in the public *Arabidopsis* EST database (Accession A042298, SEQ ID NO:38). The protein sequence (SEQ ID NO:39) was translated from the EST sequence, and was found to contain a peptide sequence conserved in both mammalian and yeast ACATs (Chang *et al.*, (1997) *Ann. Rev. Biochem.*, 66:613-638).

10 To obtain the entire coding region corresponding to the *Arabidopsis* ACAT-like EST, synthetic oligo-nucleotide primers were designed to amplify the 5' and 3' ends of partial cDNA clones containing ACAT-like sequences. Primers were designed according to the *Arabidopsis* ACAT-like EST sequence and were used in Rapid Amplification of cDNA Ends (RACE) reactions (Frohman *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002).

15 Primers were designed (5'-TGCAAATTGACGAGCACACCAACCCCTTC-3' (SEQ ID NO:40) and 5'-AAGGATGCTTGAGTCCTGACAATAGG-3' (SEQ ID NO:41)) to amplify the 5' end from the *Arabidopsis* ACAT EST sequence. Amplification of flanking sequences from cDNA clones were performed using the Marathon cDNA 20 Amplification kit (Clontech, CA).

25 The sequence derived from the 5'-RACE amplification was used to search proprietary *Arabidopsis* EST libraries. A single EST accession, LIB25-088-C7 (SEQ ID NO:42), was identified which contained a sequence identical to the 5'-RACE sequence. Furthermore, LIB25-088-C7 was found to contain the complete putative coding sequence for the *Arabidopsis* ACAT-like product.

30 The nucleic acid as well as the putative translation product sequences of A042298 were used to search public and proprietary databases. Four EST sequences were identified in both soybean (SEQ ID NOs:43-46) and maize (SEQ ID NOs:47-50) proprietary databases, and a single ACAT-like sequence was identified from *Mortierella alpina* EST sequences (SEQ ID NO:51).

Sequence alignments between ACAT sequences from several different sources were compared to identify the similarity between the sequences. Nucleotide sequences from known human and mouse ACATs, as well as nucleotide sequences from known yeast ACATs were compared to the ACAT-like EST sequences from human and *Arabidopsis*.

Analysis of the sequence alignments revealed several classes of ACATs based on sequence similarity. The known human and mouse ACATs, being 88% similar in the nucleotide sequence, formed one class of ACATs. Another class of ACATs included the yeast ACATs which are less than 20% similar to the known human and mouse class

5    ACATs.

The final class of ACATs included the Arabidopsis and human sequences disclosed in the present invention. This class is approximately 22% similar to the known human and mouse ACAT class and approximately 23% similar to the yeast class of ACATs. Thus, the ACAT sequences disclosed in the present invention represent a novel class of ACAT 10 enzymes. Partial mouse sequences of this class are also provided.

)    Example 4: Expression Construct Preparation

Constructs were prepared to direct expression of the LCAT1, LCAT2, LCAT3, LCAT4, LCAT5 and the yeast LRO1 sequences in plants and cultured insect cells. The 15 entire coding region of each LCAT was amplified from the appropriate EST clone or an Arabidopsis genomic cDNA library using the following oligonucleotide primers in a polymerase chain reactions (PCR). The LCAT1 coding sequence was amplified from the EST clone Lib25-082-Q1-E1-G4 using the primers  
5'-GGATCCGCGGCCGCACAATGAAAAAAATCTTCACATTATTCGG-3' (SEQ  
20 ID NO:52) and 5'-GGATCCCCTGCAGGTCAATTGACGGCATTACATTGG-3'  
(SEQ ID NO:53). The LCAT2 coding sequence was amplified from an Arabidopsis genomic cDNA library using the synthetic oligo nucleotide primers  
5'-GGATCCGCGGCCGCACAATGGGAGCGAATTGAAATCAGTAACG-3' (SEQ  
ID NO:54) and 5'-GGATCCCCTGCAGGTTAACCCACTTTATCAAGCTCCC-3'  
25 (SEQ ID NO:55). The LCAT3 coding sequence was amplified from the EST clone LIB22-004-Q1-E1-B4 using the synthetic oligo nucleotide primers  
5'-GGATCCGCGGCCGCACAATGTCTCTATTACTGGAA GAGATC-3' (SEQ ID  
NO:56) and 5'-GGATCCCCTGCAGGTTATGCATC AACAGAGACACTTACAGC-3'  
(SEQ ID NO:57). The LCAT4 coding sequence was amplified from the EST clone  
30 LIB23-007-Q1-E1-B5 using the synthetic oligo nucleotide primers  
5'-GGATCCGCGGCCGCACAATGGGCTGGATTCCGTGTCCGTGC-3' (SEQ ID  
NO:58) and 5'-GGATCCCCTGCAGGTTAACCCAGAATCAACTACTTG-3' (SEQ  
ID NO:59). The LCAT5 coding sequence was amplified from LIB23-053-Q1-E1-E3 using  
the synthetic oligo nucleotide primers

5'-GGATCCGCGGCCGCACAATGCCCTTATTCATCGG-3' (SEQ ID NO:77) and 5'-GGATCCCCTGCAGGTACAGCTCAGGTCAATACG-3' (SEQ ID NO:78).

The yeast LROI coding sequence was amplified from genomic yeast DNA using the synthetic oligo nucleotide primers

- 5 5'GGATCCGCGGCCGCACAATGGGCACACTGTTCGAAG3' (SEQ ID NO:79)  
and 5'GGATCCCCTGCAGGTTACATTGGGAAGGGCATCTGAG3' (SEQ ID NO:80).

The entire coding region of the *Arabidopsis* ACAT sequence (SEQ ID NO: 42) was amplified from the EST clone LIB25-088-C7 using oligonucleotide primers 5'-TCGACCTGCAGGAAGCTTAGAAATGGCGATTTGGATTC-3' (SEQ ID NO: 60).  
10 and 5'-GGATCCGCGGCCGCTCATGACATCGATCCTTTGG-3' (SEQ ID NO: 61) in a polymerase chain reaction (PCR).

Each resulting PCR product was subcloned into pCR2.1Topo (Invitrogen) and labeled pCGN9964 (LCAT1), pCGN9985 (LCAT2), pCGN9965 (LCAT3), pCGN9995 (LCAT4), pCGN10964 (LCAT5), pCGN10963 (*LROI*), and pCGN8626 (ACAT).  
15 Double stranded DNA sequence was obtained to verify that no errors were introduced by the PCR amplification.

#### 4A. Baculovirus Expression Constructs

Constructs are prepared to direct the expression of the *Arabidopsis* LCAT and yeast LCAT sequences in cultured insect cells. The entire coding region of the LCAT proteins was removed from the respective constructs by digestion with *Nos*I and *Sse*8387I, followed by gel electrophoresis and gel purification. The fragments containing the LCAT coding sequences were cloned into *Nos*I and *Pst*I digested baculovirus expression vector pFastBac1 (Gibco-BRL, Gaithersburg, MD). The resulting baculovirus expression constructs were referred to as pCGN9992 (LCAT1), pCGN9993 (LCAT2), pCGN9994 (LCAT3), pCGN10900 (LCAT4), pCGN10967 (LCAT5), and pCGN10962 (*LROI*).

#### 4B. Plant Expression Construct Preparation

A plasmid containing the napin cassette derived from pCGN3223 (described in U.S. Patent No. 5,639,790, the entirety of which is incorporated herein by reference) was modified to make it more useful for cloning large DNA fragments containing multiple restriction sites, and to allow the cloning of multiple napin fusion genes into plant binary transformation vectors. An adapter comprised of the self annealed oligonucleotide of sequence 5'-

CGCGATTAAATGGCGGCCCTGCAGGCGGCCGCCTGCAGGGCGGCCATTAA  
AAT-3' (SEQ ID NO:62) was ligated into the cloning vector pBC SK+ (Stratagene) after  
digestion with the restriction endonuclease BssHII to construct vector pCGN7765.  
Plamids pCGN3223 and pCGN7765 were digested with NotI and ligated together. The  
5 resultant vector, pCGN7770, contained the pCGN7765 backbone with the napin seed  
specific expression cassette from pCGN3223.

The cloning cassette, pCGN7787, contained essentially the same regulatory  
elements as pCGN7770, with the exception of the napin regulatory regions of pCGN7770  
have been replaced with the double CAMV 35S promoter and the tml polyadenylation and  
10 transcriptional termination region.

A binary vector for plant transformation, pCGN5139, was constructed from  
pCGN1558 (McBride and Summerfelt, (1990) Plant Molecular Biology, 14:269-276). In  
pCGN5139, the polylinker of pCGN1558 was replaced as a HindIII/Asp718 fragment with  
a polylinker containing unique restriction endonuclease sites, AscI, PacI, XbaI, Swal,  
15 BamHI, and NotI. The Asp718 and HindIII restriction endonuclease sites are retained in  
pCGN5139.

A series of turbo binary vectors was constructed to allow for the rapid cloning of  
DNA sequences into binary vectors containing transcriptional initiation regions  
(promoters) and transcriptional termination regions.

20 The plasmid pCGN8618 was constructed by ligating oligonucleotides  
5'-TCGAGGATCCGGCCGCAAGCTTCTGCAGG-3' (SEQ ID NO:63) and  
5'-TCGACCTGCAGGAAGCTTGCAGGCCGCGGATCC-3' (SEQ ID NO:64) into  
SalI/XhoI-digested pCGN7770. A fragment containing the napin promoter, polylinker and  
napin 3' region was excised from pCGN8618 by digestion with Asp718I; the fragment  
25 was blunt-ended by filling in the 5' overhangs with Klenow fragment then ligated into  
pCGN5139 that had been digested with Asp718I and HindIII and blunt-ended by filling in  
the 5' overhangs with Klenow fragment. A plasmid containing the insert oriented so that  
the napin promoter was closest to the blunted Asp718I site of pCGN5139 and the napin 3'  
was closest to the blunted HindIII site was subjected to sequence analysis to confirm both  
30 the insert orientation and the integrity of cloning junctions. The resulting plasmid was  
designated pCGN8622.

The plasmid pCGN8619 was constructed by ligating oligonucleotides  
5'-TCGACCTGCAGGAAGCTTGCAGGCCGCGGATCC-3' (SEQ ID NO:65) and

5'-TCGAGGATCCGGCGGCCGCAAGCTCCTGCAGG-3' (SEQ ID NO:66) into Sall/XhoI-digested pCGN7770. A fragment containing the napin promoter, polylinker and napin 3' region was removed from pCGN8619 by digestion with Asp718I; the fragment was blunt-ended by filling in the 5' overhangs with Klenow fragment then ligated into 5 pCGN5139 that had been digested with Asp718I and HindIII and blunt-ended by filling in the 5' overhangs with Klenow fragment. A plasmid containing the insert oriented so that the napin promoter was closest to the blunted Asp718I site of pCGN5139 and the napin 3' was closest to the blunted HindIII site was subjected to sequence analysis to confirm both the insert orientation and the integrity of cloning junctions. The resulting plasmid was 10 designated pCGN8623.

The plasmid pCGN8620 was constructed by ligating oligonucleotides 5'-TCGAGGATCCGGCGGCCGCAAGCTCCTGCAGGAGCT -3' (SEQ ID NO:67) and 5'-CCTGCAGGAAGCTTGCAGGCCGCGGATCC-3' (SEQ ID NO:68) into Sall/SacI-digested pCGN7787. A fragment containing the d35S promoter, polylinker and tml 3' 15 region was removed from pCGN8620 by complete digestion with Asp718I and partial digestion with NotI. The fragment was blunt-ended by filling in the 5' overhangs with Klenow fragment then ligated into pCGN5139 that had been digested with Asp718I and HindIII and blunt-ended by filling in the 5' overhangs with Klenow fragment. A plasmid containing the insert oriented so that the d35S promoter was closest to the blunted 20 Asp718I site of pCGN5139 and the tml 3' was closest to the blunted HindIII site was subjected to sequence analysis to confirm both the insert orientation and the integrity of cloning junctions. The resulting plasmid was designated pCGN8624.

The plasmid pCGN8621 was constructed by ligating oligonucleotides 5'-TCGACCTGCAGGAAGCTTGCAGGCCGCGGATCCAGCT -3' (SEQ ID NO:69) and 25 5'-GGATCCGGCCGCAAGCTCCTGCAGG-3' (SEQ ID NO:70) into Sall/SacI-digested pCGN7787. A fragment containing the d35S promoter, polylinker and tml 3' region was removed from pCGN8621 by complete digestion with Asp718I and partial digestion with NotI. The fragment was blunt-ended by filling in the 5' overhangs with Klenow fragment then ligated into pCGN5139 that had been digested with Asp718I and 30 HindIII and blunt-ended by filling in the 5' overhangs with Klenow fragment. A plasmid containing the insert oriented so that the d35S promoter was closest to the blunted Asp718I site of pCGN5139 and the tml 3' was closest to the blunted HindIII site was subjected to sequence analysis to confirm both the insert orientation and the integrity of cloning junctions. The resulting plasmid was designated pCGN8625.

The plasmid construct pCGN8640 is a modification of pCGN8624 described above. A 938bp PstI fragment isolated from transposon Tn7 which encodes bacterial spectinomycin and streptomycin resistance (Fling et al. (1985), *Nucleic Acids Research* 13(19):7095-7106), a determinant for *E. coli* and Agrobacterium selection, was blunt ended with Pfu polymerase. The blunt ended fragment was ligated into pCGN8624 that had been digested with SpeI and blunt ended with Pfu polymerase. The region containing the PstI fragment was sequenced to confirm both the insert orientation and the integrity of cloning junctions.

5 The spectinomycin resistance marker was introduced into pCGN8622 and pCGN8623 as follows. A 7.7 Kbp AvrII-SnaBI fragment from pCGN8640 was ligated to 10 a 10.9 Kbp AvrII-SnaBI fragment from pCGN8623 or pCGN8622, described above. The resulting plasmids were pCGN8641 and pCGN8643, respectively.

) The plasmid pCGN8644 was constructed by ligating oligonucleotides 15 5'-GATCACCTGCAGGAAGCTTGCGGCCGCGATCCAATGCA-3' (SEQ ID NO:71) and 5'-TTGGATCCGCGGCCGCAAGCTTCCTGCAGGT-3' (SEQ ID NO:72) into BamHI-PstI digested pCGN8640.

#### 4C. Plant LCAT Expression Construct Preparation

The coding sequence of LCAT1 was cloned from pCGN9964 as a *NotI/Sse8387I* 20 fragment into pCGN8640, pCGN8641, pCGN8643, and pCGN8644 to create the expression constructs pCGN9960, pCGN9961, pCGN9962, and pCGN9963, respectively. The construct pCGN9960 was designed to express the LCAT1 coding sequence in the sense orientation from the constitutive promoter CaMV 35S. The construct pCGN9961 25 was designed to express the LCAT1 coding sequence in the antisense orientation from the napin promoter. The construct pCGN9962 was designed to express the LCAT1 coding sequence in the sense orientation from the napin promoter. The construct pCGN9963 was designed to express the LCAT1 coding sequence in the antisense orientation from the constitutive promoter CaMV 35S.

The coding sequence of LCAT2 was cloned from pCGN9985 as a *NotI/Sse8387I* 30 fragment into pCGN8640, pCGN8641, pCGN8643, and pCGN8644 to create the expression constructs pCGN9981, pCGN9982, pCGN9983, and pCGN9984, respectively. The construct pCGN9981 was designed to express the LCAT2 coding sequence in the sense orientation from the constitutive promoter CaMV 35S. The construct pCGN9982 was designed to express the LCAT2 coding sequence in the antisense orientation from the

napin promoter. The construct pCGN9983 was designed to express the LCAT2 coding sequence in the sense orientation from the napin promoter. The construct pCGN9984 was designed to express the LCAT2 coding sequence in the antisense orientation from the constitutive promoter CaMV 35S.

- 5       The coding sequence of LCAT3 was cloned from pCGN9965 as a *NotI/Sse8387I* fragment into pCGN8640, pCGN8641, pCGN8643, and pCGN8644 to create the expression constructs pCGN9966, pCGN9967, pCGN9968, and pCGN9969, respectively. The construct pCGN9966 was designed to express the LCAT3 coding sequence in the sense orientation from the constitutive promoter CaMV 35S. The construct pCGN9967  
10      was designed to express the LCAT3 coding sequence in the antisense orientation from the napin promoter. The construct pCGN9968 was designed to express the LCAT3 coding sequence in the sense orientation from the napin promoter. The construct pCGN9969 was designed to express the LCAT3 coding sequence in the antisense orientation from the constitutive promoter CaMV 35S.
- 15      The coding sequence of LCAT4 was cloned from pCGN9995 as a *NotI/Sse8387I* fragment into pCGN8640, pCGN8641, pCGN8643, and pCGN8644 to create the expression constructs pCGN9996, pCGN9997, pCGN9998, and pCGN9999, respectively. The construct pCGN9996 was designed to express the LCAT4 coding sequence in the sense orientation from the constitutive promoter CaMV 35S. The construct pCGN9997  
20      was designed to express the LCAT4 coding sequence in the antisense orientation from the napin promoter. The construct pCGN9998 was designed to express the LCAT4 coding sequence in the sense orientation from the napin promoter. The construct pCGN9999 was designed to express the LCAT4 coding sequence in the antisense orientation from the constitutive promoter CaMV 35S.
- 25      The coding sequence of LCAT5 was cloned from pCGN10964 as a *NotI/Sse8387I* fragment into pCGN9977 and pCGN9979, to create the expression constructs pCGN10965, and pCGN10966, respectively. The construct pCGN10965 was designed to express the LCAT5 coding sequence in the sense orientation from the constitutive promoter CaMV 35S. The construct pCGN10966 was designed to express the LCAT5 coding sequence in the sense orientation from the napin promoter.

The coding sequence of *LRO1* was cloned from pCGN10963 as a *NotI/Sse8387I* fragment into pCGN9977 and pCGN9979, to create the expression constructs pCGN10960, and pCGN10961, respectively. The construct pCGN10960 was designed to express the *LRO1* coding sequence in the sense orientation from the constitutive promoter

CaMV 35S. The construct pCGN10961 was designed to express the *LRO1* coding sequence in the sense orientation from the napin promoter.

#### 4D. Plant ACAT Expression Construct Preparation

5 A fragment containing the *Arabidopsis* ACAT-like coding region was removed from pCGN8626 by digestion with Sse8387I and Not I. The fragment containing the ACAT-like sequence was ligated into PstI-Not I digested pCGN8622. The resulting plasmid was designated pCGN8627. DNA sequence analysis confirmed the integrity of the cloning junctions.

10 A fragment containing the *Arabidopsis* ACAT-like coding region (SEQ ID NO: 42) was removed from pCGN8626 by digestion with Sse8387I and Not I. The fragment was ligated into PstI-Not I digested pCGN8623. The resulting plasmid was designated pCGN8628. DNA sequence analysis confirmed the integrity of the cloning junctions.

15 A fragment containing the *Arabidopsis* ACAT-like coding region was removed from pCGN8626 by digestion with Sse8387 and Not I. The fragment was ligated into PstI-Not I digested pCGN8624. The resulting plasmid was designated pCGN8629. DNA sequence analysis confirmed the integrity of the cloning junctions.

20 A fragment containing the *Arabidopsis* ACAT-like coding region was removed from pCGN8626 by digestion with Sse8387 and Not I. The fragment was ligated into PstI-Not I digested pCGN8625. The resulting plasmid was designated pCGN8630. DNA sequence analysis confirmed the integrity of the cloning junctions.

25 An additional expression construct for the suppression of endogenous ACAT-like activity was also prepared. The construct pCGN8660 was constructed by cloning approximately 1 Kb of the *Arabidopsis* ACAT-like coding region from pCGN8626 in the sense orientation, and the full-length *Arabidopsis* ACAT-like coding region in the antisense orientation under the regulatory control of the napin transcription initiation sequence.

For expression of the rat ACAT-like sequence in plants, the NotI-Sse8387I fragment of pCGN8592 was cloned into NotI-PstI digested binary vectors pCGN8621, 30 pCGN8622, and pCGN8624 to yield plasmids, pCGN 9700, pCGN9701, and pCGN9702, respectively. Plasmid pCGN9700 expresses a sense transcript of the rat ACAT-like cDNA under control of a napin promoter, plasmid pCGN9701 expresses an antisense transcript of the rat ACAT-like cDNA under control of a napin promoter, and plasmid pCGN9702 expresses a sense transcript of the rat ACAT-like cDNA under control of a double 35S

promoter. Plasmids pCGN 9700, pCGN9701, and pCGN9702 were introduced in *Agrobacterium tumefaciens* EHA101.

Constructs were prepared to direct the expression of the rat ACAT-like sequence in the seed embryo of soybean and the endosperm of corn. For expression of the rat ACAT-like DNA sequence in soybean, a 1.5 kb *NotI/Sse8387I* fragment from pCGN8592 containing the coding sequence of the rat ACAT-like sequence was blunt ended using Mung bean nuclease, and ligated into the *SmaI* site of the turbo 7S binary/cloning vector pCGN8809 to create the vector pCGN8817 for transformation into soybean by particle bombardment. The vector pCGN8817 contained the operably linked components of the promoter region of the soybean  $\alpha'$  subunit of  $\beta$ -conglycinin (7S promoter, (Chen *et al.*, 1986), *Proc. Natl. Acad. Sci.*, 83:8560-8564), the DNA sequence coding for the entire rat ACAT-like protein, and the transcriptional termination region of pea RuBisCo small subunit, referred to as E9 3' (Coruzzi, *et al.* (1984) *EMBO J.* 3:1671-1679 and Morelli, *et al.* (1985) *Nature* 315:200-204). This construct further contained sequences for the selection of positive transformed plants by screening for resistance to glyphosate using the CP4 EPSPS (U.S. Patent 5,633,435) expressed under the control of the figwort mosaic virus (FMV) promoter (U.S. Patent Number 5,378,619) and the transcriptional termination region of E9.

For expression of the rat ACAT-like sequence in the corn endosperm, a 1.5 kb *NotI/Sse8387I* fragment from pCGN8592 containing the coding sequence of the rat ACAT-like sequence was blunt ended using Mung bean nuclease, and ligated into the *BamHI* site of the rice pGt1 expression cassette pCGN8592 for expression from the pGt1 promoter (Leisy, D.J. *et al.*, *Plant Mol. Biol.* 14 (1989) 41-50) and the HSP70 intron sequence (U.S. Patent Number 5,593,874). This cassette also included the transcriptional termination region downstream of the cloning site of nopaline synthase, *nos* 3' (Depicker *et al.*, *J. Molec. Appl. Genet.* (1982) 1: 562-573). A 7.5 kb fragment containing the pGt1 promoter, the DNA sequence encoding the rat ACAT-like protein, and the *nos* transcriptional termination sequence was cloned into the binary vector pCGN8816 to create the vector pCGN8818 for transformation into corn. This construct also contained sequences for the selection of positive transformants with kanamycin using the kanamycin resistance gene from Tn5 bacteria under the control of the CAMV 35S promoter and tml transcriptional termination regions.

**Example 5: Expression in Insect Cell Culture**

A baculovirus expression system was used to express the LCAT cDNAs in cultured insect cells.

- The baculovirus expression constructs pCGN9992, pCGN9993, pCGN9994, 5 pCGN10900, pCGN10962, and pCGN10967 were transformed and expressed using the BAC-to-BAC Baculovirus Expression System (Gibco-BRL, Gaithersburg, MD) according to the manufacturer's directions.

The transformed insect cells were used to assay for acyltransferase activities using methods known in the art (see Example 8).

10

**Example 6: Plant Transformation**

- A variety of methods have been developed to insert a DNA sequence of interest into the genome of a plant host to obtain the transcription or transcription and translation of the sequence to effect phenotypic changes. Transgenic plants were obtained by *Agrobacterium*-mediated transformation as described by Radke *et al.* (*Theor. Appl. Genet.* (1988) 75:685-694; 15 *Plant Cell Reports* (1992) 11:499-505). Alternatively, microprojectile bombardment methods, such as described by Klein *et al.* (*Bio/Technology* 10:286-291) may also be used to obtain nuclear transformed plants. Other plant species may be similarly transformed using related techniques.
- 20 The plant binary constructs described above were used in plant transformation to direct the expression of the sterol acyltransferases in plant tissues. Binary vector constructs were transformed into strain EHA101 *Agrobacterium* cells (Hood *et al.*, *J. Bacteriol.* (1986) 168:1291-1301), by the method of Holsters *et al.* (*Mol. Gen. Genet.* (1978) 163:181-187). Transgenic *Arabidopsis thaliana* plants were obtained by *Agrobacterium*-mediated 25 transformation as described by Valverkens *et al.*, (*Proc. Nat. Acad. Sci.* (1988) 85:5536-5540), Bent *et al.* ((1994), *Science* 265:1856-1860), and Bechtold *et al.* ((1993), *C. R. Acad. Sci., Life Sciences* 316:1194-1199).

**Example 7: Plant Assays for Modified Sterol Content/Profile**

- 30 7A: NMR of T2 seed

Seed from plants expressing LCAT 1 through 4 under the control of the napin promoter were analyzed by NMR. *Arabidopsis* seeds from transgenic plants were placed directly into wide-mouth MAS NMR sample tubes.

High-resolution spectra were measured at 11.7 T (1H=500 MHz, 13C=125 mHz) using Varian NMR Instruments (Palo Alto, CA) Inova™ NMR spectrometers equipped with carbon-observe MAS Nanoprobes™. The 13C spectra were acquired without a field-frequency lock at ambient temperature (approx. 21-22°C) for 14 hours using the following conditions: spectral width = 29.996 kHz, acquisition time = 2.185 seconds, p/2 pulse (3.8 ms) with no relaxation delay, 1H g B2 = 2.5 kHz with Waltz decoupling. Data processing conditions were typically: digital resolution = 0.11 Hz, 0.3 to 1.5 Hz line broadening and time-reversed linear prediction of the first three data points. Chemical shifts were referenced by adding neat tetramethylsilane (TMS) to *Arabidopsis* seeds and using the resulting referencing parameters for subsequent spectra. The 13C resolution was 2-3 Hz for the most narrow seed resonances. Spectral resolution was independent of MAS spinning speeds (1.5-3.5 kHz) and data were typically obtained with 1.5 kHz spinning speeds. Spinning sidebands were approx. 1% of the main resonance. Phytosterol 13C assignments were based on model samples composed of triolein,  $\beta$ -sitosterol and cholesterol oleate. Triacylglycerol 13C assignments were made from comparison with literature assignments or with shifts computed from a 13C NMR database (Advanced Chemical Development, Inc., version 3.50, Toronto Canada).

The results of these analyses are displayed in Figure 2 and show that there was a trend of an approximately 2 fold increase of phytosterols in the seeds derived from plant line 5 expressing the LCAT 4 gene (pCGN9998) under the control of the napin promoter. During the course of this analysis it was also noted that the average oil content of seed from plants expressing the LCAT2 construct (pCGN9983) under the control of the napin promoter was higher than that of controls. This is the first *in planta* evidence supporting the concept that overexpression of a nucleotide sequence encoding a lecithin:cholesterol acyltransferase-like polypeptide can increase oil content.

#### 7B: HPLC/MS of T2 seed

Seed oil from T2 plants expressing LCAT1 through 4 under the control of the napin promoter was extracted using an accelerated solvent extractor (ASE) method. Seed samples were ground, using a mortar and pestle, to achieve a fine homogeneous meal. Oil was obtained using a Dionex Accelerated Solvent Extractor (ASE). Clean ground seed was added to an equal amount of diatomaceous earth. The ground seed sample and the diatomaceous earth were thoroughly mixed until a homogeneous texture was achieved.

The sample was then loaded into the instrument and oil extraction was achieved using hexane under validated laboratory protocols.

Oil from these seed samples was then analyzed for sterol ester analysis using HPLC/MS for free campesterol, stigmasterol, and sitosterol and their fatty acid esters. To 5 the autosampler vial containing approximately 0.1 grams oil was added 0.3 mLs CDCl<sub>3</sub>. One-hundred microliters of this solution was added to 900 microliters CHCl<sub>3</sub>. Five microliters of this diluted sample was subsequently injected into an HPLC/MS with positive ion atmospheric pressure ionization. The individual components in the oils were separated using two 4.6 x 50 mm C<sub>8</sub> Zorbax columns in series and a gradient using 10 acetonitrile and acetonitrile with 40% CHCl<sub>3</sub>. The sterol concentrations were calculated assuming each sterol and its fatty acids have the same molar responses. This was observed to be the case with cholesterol and its esters and was assumed to be the case for campesterol, stigmasterol, and sitosterol. In the present study, the sterol identified as stigmasterol was actually an isomer of this compound.

15 The results of these analyses are displayed in Figures 3 and 4 and show that there were sterol ester enhancements on the order of 50% in the seeds derived from six out of seven T2 plant lines expressing LCAT3 (pCGN9968) under the control of the napin promoter.

20 **Example 8: Baculovirus Insect Cell Culture for Sterol Esterification Activity**

Baculovirus expression construct pCGN9992, pCGN9993, pCGN9994 and pCGN10900 (see Example 4) were transformed and expressed using the BAC-TO-BAC Baculovirus Expression System (Gibco-BRL, Gaithersburg, MD) according to the manufacturer's instructions except harvesting of recombinant viruses was done 5 days 25 post-transfection. The supernatant from the transfection mixture was used for generating virus stock which in turn was used for infecting Sf9 cells used in the assay.

The transformed cells were assayed for lecithin:sterol acyltransferase activities using the method described herein. Insect cells were centrifuged and the resulting cell pellet was either used immediately or stored at -70 C for later analysis. Cells were 30 resuspended in Medium A (100 mM Tricine/NaOH, pH 7.8, 10% (w/v) glycerol, 280 mM NaCl with : 0.1 µM Aprotinin, 1 µM Leupeptin, and 100 µM Pefabloc (all from Boehringer Mannheim, Germany) and lysed by sonication (2 x 10 sec). Cell walls and other debris were pelleted by centrifugation (14,000 x g, 10 min, 4°C). The supernatant

was transferred to a new vial and membranes pelleted by centrifugation (100,000 x g, Ti 70.1 rotor, 46,000 rpm for 1 hour at 4°C). Total membranes were resuspended in Medium A. Lecithin:sterol acyltransferase activity was assayed in a 0.1 ml reaction mixture containing 100 mM Tris/HCl, pH 7, 28 mM NaCl, 0.03% Triton X-100, 0.1 mM sitosterol, 5 20 µM 1,2-[<sup>14</sup>C]-palmitoyl-phosphatidyl choline (246420 dpm/nmole), and 0.05-20 mg of membrane protein. After 15 minutes at 30 °C, the reaction was terminated by addition of a 0.5 ml solution of methylene chloride:methanol (4:1, v/v) containing 100 µg cholesterol and cholesterol ester as cold carriers. A portion (0.1 ml) of the bottom organic layer was removed and evaporated under nitrogen gas. The concentrated extract was resuspended in 10 30 µl of hexane and spotted onto a silica gel-G thin layer chromatographic plate. The plate was migrated in hexane:diethyl ether:acetic acid (80:20:1) to the top, then air dried. Radioactivity was determined by exposure to a Low Energy Phosphor-imaging Screen. Following exposure, the screen was read on a phosphorimager.

The LCAT 4 protein from pCGN10900 in baculovirus membranes showed a 15 radioactive spot in the region of the TLC plate where cholesterol ester migrates indicating that LCAT 4 has the ability to catalyze the transfer of an acyl group from lecithin (PC) to sitosterol to make a sitosterol ester.

#### Example 9: Plant Assay for Modified Lipid Content

Nir (near infrared spectroscopy spectral scanning) can be used to determine the total oil content of Arabidopsis seeds in a non-destructive way provided that a spectral calibration curve has been developed and validated for seed oil content. A seed oil spectral calibartion curve was developed using seed samples from 85 Arabidopsis plants. Seed was cleaned and scanned using a Foss NIR model 6500 (Foss-Nirs Systems, Inc.). 20 25 Approximately 50 to 100 milligrams of whole seeds, per sample, were packed in a mini sample ring cup with quartz lens [ IH-0307 ] consisting a mini-insert [ IH-0337 ] and scanned in reflectance mode to obtain the spectral data. The seed samples were then ground, using a mortar and pestle, to achieve a fine homogeneous meal. The ground samples were measured for oil using an accelerated solvent extractor (ASE).

Measurement for the total oil content was performed on the Dionex Accelerated Solvent Extractor (ASE). Approximately 500 mg of clean ground seed was weighed to the nearest 0.1 mg onto a 9 x 9 cm weigh boat. An equal amount of diatomaceous earth was added using a top-loading balance accurate to the nearest 0.01 g. The ground seed sample 30

and the diatomaceous earth were thoroughly mixed until a homogeneous texture was achieved. The sample was loaded on to the instrument and oil extraction was achieved using hexane under validated laboratory protocols. Standard Rapeseed samples were obtained from the Community Bureau of Reference (BCR). The ASE extraction method 5 was validated using the BCR reference standards. A total percent oil recovery of 99% to 100% was achieved. "As-is" oil content was calculated to the nearest 0.01 mass percentage using the formula:

$$\text{Oil Content} = 100\% \times (\text{vial plus extracted oil wt} - \text{initial vial wt}) / (\text{sample wt})$$

10

) The analytical data generated by ASE were used to perform spectral calibrations. Nir calibration equations were generated using the built-in statistical package within the NirSystems winisi software. The spectral calibration portion of the software is capable of calibration and self-validation. From a total of 85 samples, 57 samples were used to 15 generate the total percent oil calibration. The remaining samples were used to validate the oil calibrations. Optimized smoothing, derivative size, and mathematical treatment (modified partial least square) was utilized to generate the calibration. The samples that were not used in building respective calibrations were used as a validation set. Statistical tools such as correlation coefficient ( R ), coefficient of determination ( R<sup>2</sup> ), standard error 20 of prediction ( SEP ), and the standard error of prediction corrected for bias ( SEPC ) were used to evaluate the calibration equations.

) T2 seeds from plants that had been transformed with the LCAT genes were cleaned and scanned using a Foss NIR model 6500 (Foss-Nirs Systems, Inc.). Approximately 50 to 100 milligrams of whole seeds, per sample, were packed in a mini sample ring cup with 25 quartz lens [ IH-0307 ] consisting a mini-insert [ IH-0337 ] and scanned in reflectance mode to obtain the spectral data. Oil percentage in each seed sample was determined using the seed oil spectral calibration curve detailed above.

The results of these analyses are found in Figure 5 and Table 2 and show that there was a significant increase in the oil level in seed from T2 plants expressing the LCAT2 30 gene. This increase in oil was seen in plants when LCAT2 was driven by either the 35S constitutive promoter or the seed-specific napin promoter. These results show that overexpression of a nucleic acid sequence encoding a lecithin:cholesterol acyltransferase-like polypeptide can increase seed oil production in plants.

Table 2

|             | Construct number | Seed Oil Percentage (%) |
|-------------|------------------|-------------------------|
| CONTROL     |                  | 24.7                    |
| CONTROL     |                  | 28.0                    |
| CONTROL     |                  | 31.8                    |
| CONTROL     |                  | 32.4                    |
| NAPIN LCAT1 | PCGN9962         | 28.5                    |
| NAPIN LCAT1 | PCGN9962         | 28.9                    |
| NAPIN LCAT1 | PCGN9962         | 29.6                    |
| NAPIN LCAT1 | PCGN9962         | 30.1                    |
| NAPIN LCAT1 | PCGN9962         | 30.1                    |
| NAPIN LCAT1 | PCGN9962         | 30.1                    |
| NAPIN LCAT1 | PCGN9962         | 30.8                    |
| NAPIN LCAT1 | PCGN9962         | 31.0                    |
| NAPIN LCAT1 | pCGN9962         | 32.1                    |
| NAPIN LCAT1 | pCGN9962         | 34.2                    |
| NAPIN LCAT3 | pCGN9968         | 26.8                    |
| NAPIN LCAT3 | pCGN9968         | 27.4                    |
| NAPIN LCAT3 | pCGN9968         | 29.0                    |
| NAPIN LCAT3 | pCGN9968         | 29.0                    |
| NAPIN LCAT3 | pCGN9968         | 32.6                    |
| NAPIN LCAT2 | pCGN9983         | 26.5                    |
| NAPIN LCAT2 | pCGN9983         | 34.7                    |
| NAPIN LCAT2 | pCGN9983         | 34.8                    |
| NAPIN LCAT2 | pCGN9983         | 35.7                    |
| NAPIN LCAT2 | pCGN9983         | 35.8                    |
| NAPIN LCAT2 | pCGN9983         | 36.3                    |
| NAPIN LCAT2 | pCGN9983         | 36.7                    |
| NAPIN LCAT2 | pCGN9983         | 37.0                    |
| NAPIN LCAT2 | pCGN9983         | 37.2                    |
| NAPIN LCAT2 | pCGN9983         | 37.3                    |
| NAPIN LCAT2 | pCGN9983         | 37.3                    |
| NAPIN LCAT2 | pCGN9983         | 37.4                    |
| NAPIN LCAT2 | pCGN9983         | 37.8                    |
| NAPIN LCAT2 | pCGN9983         | 38.0                    |
| NAPIN LCAT2 | pCGN9983         | 38.0                    |
| 35S LCAT2   | pCGN9981         | 27.3                    |
| 35S LCAT2   | pCGN9981         | 28.1                    |
| 35S LCAT2   | pCGN9981         | 28.2                    |
| 35S LCAT2   | pCGN9981         | 28.6                    |
| 35S LCAT2   | pCGN9981         | 29.8                    |
| 35S LCAT2   | pCGN9981         | 30.3                    |
| 35S LCAT2   | pCGN9981         | 32.4                    |
| 35S LCAT2   | pCGN9981         | 32.5                    |
| 35S LCAT2   | pCGN9981         | 33.6                    |
| 35S LCAT2   | pCGN9981         | 34.1                    |
| 35S LCAT2   | pCGN9981         | 35.5                    |
| 35S LCAT2   | pCGN9981         | 36.4                    |
| 35S LCAT2   | pCGN9981         | 37.1                    |
| 35S LCAT2   | pCGN9981         | 38.3                    |

|           |          |      |
|-----------|----------|------|
| 35S LCAT2 | pCGN9981 | 38.5 |
| 35S LCAT2 | pCGN9981 | 39.1 |

In light of the detailed description of the invention and the examples presented above, it can be appreciated that the several aspects of the invention are achieved.

- 5 It is to be understood that the present invention has been described in detail by way of illustration and example in order to acquaint others skilled in the art with the invention, its principles, and its practical application. Particular formulations and processes of the present invention are not limited to the descriptions of the specific embodiments presented, but rather the descriptions and examples should be viewed in terms of the
- ) 10 claims that follow and their equivalents. While some of the examples and descriptions above include some conclusions about the way the invention may function, the inventors do not intend to be bound by those conclusions and functions, but put them forth only as possible explanations.

It is to be further understood that the specific embodiments of the present invention  
15 as set forth are not intended as being exhaustive or limiting of the invention, and that many alternatives, modifications, and variations will be apparent to those of ordinary skill in the art in light of the foregoing examples and detailed description. Accordingly, this invention is intended to embrace all such alternatives, modifications, and variations that fall within the spirit and scope of the following claims.

What is claimed is:

1. An isolated nucleic acid sequence comprising a polynucleotide encoding a plant lecithin:cholesterol acyltransferase-like polypeptide or fragment thereof.
2. The isolated nucleic acid sequence of claim 1, wherein said plant lecithin:cholesterol acyltransferase-like polypeptide is selected from the group consisting of *Arabidopsis*, soybean and corn.
3. An isolated nucleic acid sequence comprising a polynucleotide encoding a plant acyl CoA:cholesterol acyltransferase-like polypeptide.
4. The isolated nucleic acid sequence of claim 3, wherein said polynucleotide is SEQ ID NO: 42 or degenerate variants thereof.
5. The isolated nucleic acid sequence of claim 1, wherein said polynucleotide is selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 43, 44, 45, 46, 47, 48, 49, 50, 51, 73 and 75 or degenerate variants thereof.
6. An isolated nucleic acid sequence consisting essentially of SEQ ID NO: 2, 4, 6, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 73 or 75.
7. An isolated nucleic acid sequence consisting of SEQ ID NO: 2, 4, 6, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 73 or 75.
8. An isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of:
  - a) an isolated polynucleotide encoding a polypeptide of SEQ ID NO 3 or SEQ ID NO 3 with at least one conservative amino acid substitution;
  - b) SEQ ID NO: 2;
  - c) an isolated polynucleotide that has at least 70% sequence identity to SEQ ID NO: 2;

- d) an isolated polynucleotide that has at least 80% sequence identity to SEQ ID NO: 2;
- 10 e) an isolated polynucleotide that has at least 90% sequence identity to SEQ ID NO: 2;
- f) an isolated polynucleotide that has at least 95% sequence identity to SEQ ID NO: 2;
- 15 g) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 2;
- h) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d), (e), (f) or (g); and
- i) an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 2 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.

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9. An isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3', where X is hydrogen, Y is hydrogen or a metal, R<sub>1</sub> and R<sub>3</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>2</sub> is selected from the group consisting of:
- 5 a) an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 3 or SEQ ID NO: 3 with at least one conservative amino acid substitution;
  - b) SEQ ID NO: 2;
  - c) an isolated polynucleotide that has at least 70% sequence identity to SEQ ID NO: 2;
  - 10 d) an isolated polynucleotide that has at least 80% sequence identity to SEQ ID NO: 2;
  - e) an isolated polynucleotide that has at least 90% sequence identity to SEQ ID NO: 2;
  - f) an isolated polynucleotide that has at least 95% sequence identity to SEQ ID NO: 2;
  - 15 g) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 2;
  - h) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d), (e), (f) or (g); and

- 20           i)     an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 2 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.
10.   An isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of:
- 5           a)    an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 5 or SEQ ID NO: 5 with at least one conservative amino acid substitution;
- b)    SEQ ID NO: 4;
- c)    an isolated polynucleotide having at least 70% sequence identity with SEQ ID NO: 4;
- d)    an isolated polynucleotide having at least 80% sequence identity with SEQ ID NO: 4;
- 10          e)    an isolated polynucleotide having at least 90% sequence identity with SEQ ID NO: 4;
- f)    an isolated polynucleotide having at least 95% sequence identity with SEQ ID NO: 4;
- 15          g)    an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 4;
- h)    an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d), (e), (f) or (g); and
- i)    an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 4 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.
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11.   An isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3', where X is hydrogen, Y is hydrogen or a metal, R<sub>1</sub> and R<sub>3</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>2</sub> is selected from the group consisting of:
- 5           a)    an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 5 or SEQ ID NO: 5 with at least one conservative amino acid substitution;
- b)    SEQ ID NO: 4;

- c) an isolated polynucleotide having at least 70% sequence identity with SEQ ID NO: 4;
  - 10 d) an isolated polynucleotide having at least 80% sequence identity with SEQ ID NO: 4;
  - e) an isolated polynucleotide having at least 90% sequence identity with SEQ ID NO: 4;
  - 15 f) an isolated polynucleotide having at least 95% sequence identity with SEQ ID NO: 4;
  - g) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 4;
  - h) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c),  
) (d), (e), (f) or (g); and
  - 20 i) an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 4 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.
12. An isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of:
- a) an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 7 or SEQ ID NO: 7 with at least one conservative amino acid substitution;
  - 5 b) SEQ ID NO: 6;
  - c) an isolated polynucleotide having at least 70% sequence identity with SEQ ID NO: 6;
  - d) an isolated polynucleotide having at least 80% sequence identity with SEQ ID NO: 6;
  - 10 e) an isolated polynucleotide having at least 90% sequence identity with SEQ ID NO: 6;
  - f) an isolated polynucleotide having at least 95% sequence identity with SEQ ID NO: 6;
  - 15 g) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 6;
  - h) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d), (e), (f) or (g); and

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- i) an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 6 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.
13. An isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3', where X is hydrogen, Y is hydrogen or a metal, R<sub>1</sub> and R<sub>3</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>2</sub> is selected from the group consisting of:
  - a) an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 7 or SEQ ID NO: 7 with at least one conservative amino acid substitution;
  - b) SEQ ID NO: 6;
  - c) an isolated polynucleotide having at least 70% sequence identity with SEQ ID NO: 6;
  - 10 d) an isolated polynucleotide having at least 80% sequence identity with SEQ ID NO: 6;
  - e) an isolated polynucleotide having at least 90% sequence identity with SEQ ID NO: 6;
  - f) an isolated polynucleotide having at least 95% sequence identity with SEQ ID NO: 6;
  - 15 g) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 6;
  - h) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d), (e), (f) or (g); and
- 20 i) an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 6 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.
14. An isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of
  - a) an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 9 or SEQ ID NO: 9 with at least one conservative amino acid substitution;
  - 5 b) SEQ ID NO 8;

- c) an isolated polynucleotide having at least 70% sequence identity with SEQ ID NO: 8;
- d) an isolated polynucleotide having at least 80% sequence identity with SEQ ID NO: 8;
- 10 e) an isolated polynucleotide having at least 90% sequence identity with SEQ ID NO: 8;
- f) an isolated polynucleotide having at least 95% sequence identity with SEQ ID NO: 8;
- 15 g) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 8;
- h) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d),  
) (e), (f) or (g); and
- i) an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 8 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.

15. An isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3', where X is hydrogen, Y is hydrogen or a metal, R<sub>1</sub> and R<sub>3</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>2</sub> is selected from the group consisting of:

- 5 a) an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 9 or SEQ ID NO: 9 with at least one conservative amino acid substitution;
- b) SEQ ID NO: 8;
- c) an isolated polynucleotide having at least 70% sequence identity with SEQ ID NO: 8;
- 10 d) an isolated polynucleotide having at least 80% sequence identity with SEQ ID NO: 8;
- e) an isolated polynucleotide having at least 90% sequence identity with SEQ ID NO: 8;
- f) an isolated polynucleotide having at least 95% sequence identity with SEQ ID NO: 8;
- 15 g) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 8;

- 20 h) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d), (e), (f) or (g); and  
i) an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 8 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.

16. An isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of:  
a) an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 74 or SEQ ID NO: 74 with at least one conservative amino acid substitution;  
5 b) SEQ ID NO: 73;  
c) an isolated polynucleotide having at least 70% sequence identity with SEQ ID NO: 73;  
d) an isolated polynucleotide having at least 80% sequence identity with SEQ ID NO: 73;  
10 e) an isolated polynucleotide having at least 90% sequence identity with SEQ ID NO: 73;  
f) an isolated polynucleotide having at least 95% sequence identity with SEQ ID NO: 73;  
g) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 73;  
15 h) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d), (e), (f) or (g); and  
i) an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 73 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.  
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17. An isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3', where X is hydrogen, Y is hydrogen or a metal, R<sub>1</sub> and R<sub>3</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>2</sub> is selected from the group consisting of:  
5 a) an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 74 or SEQ ID NO: 74 with at least one conservative amino acid substitution;

- b) SEQ ID NO: 73;
- c) an isolated polynucleotide having at least 70% sequence identity with SEQ ID NO: 73;
- 10 d) an isolated polynucleotide having at least 80% sequence identity with SEQ ID NO: 73;
- e) an isolated polynucleotide having at least 90% sequence identity with SEQ ID NO: 73;
- f) an isolated polynucleotide having at least 95% sequence identity with SEQ ID NO: 73;
- 15 g) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 73;
- ) h) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d), (e), (f) or (g); and
- 20 i) an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 73 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.

18. A isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of:
- a) an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 76 or SEQ ID NO: 76 with at least one conservative amino acid substitution;
  - 5 b) SEQ ID NO: 75;
  - c) an isolated polynucleotide having at least 70% sequence identity with SEQ ID NO: 75;
  - d) an isolated polynucleotide having at least 80% sequence identity with SEQ ID NO: 75;
  - 10 e) an isolated polynucleotide having at least 90% sequence identity with SEQ ID NO: 75;
  - f) an isolated polynucleotide having at least 95% sequence identity with SEQ ID NO: 75;
  - 15 g) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 75;

- 20 h) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d), (e), (f) or (g); and
- i) an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 75 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.
19. An isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3', where X is hydrogen, Y is hydrogen or a metal, R<sub>1</sub> and R<sub>3</sub> are any nucleic acid, n is an integer between 0-3000, and R<sub>2</sub> is selected from the group consisting of:
- 5 a) an isolated polynucleotide encoding a polypeptide of SEQ ID NO: 76 or SEQ ID NO: 76 with at least one conservative amino acid substitution;
- b) SEQ ID NO: 75;
- c) an isolated polynucleotide having at least 70% sequence identity with SEQ ID NO: 75;
- 10 d) an isolated polynucleotide having at least 80% sequence identity with SEQ ID NO: 75;
- e) an isolated polynucleotide having at least 90% sequence identity with SEQ ID NO: 75;
- f) an isolated polynucleotide having at least 95% sequence identity with SEQ ID NO: 75;
- 15 g) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 75;
- h) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d), (e), (f) or (g); and
- 20 i) an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 75 and encodes a plant lecithin:cholesterol acyltransferase-like polypeptide.
20. An isolated nucleic acid sequence comprising a polynucleotide selected from the group consisting of:
- a) SEQ ID NO: 42 or a degenerate variant thereof;

- b) an isolated polynucleotide having at least 70% sequence identity with SEQ ID NO: 42;
- c) an isolated polynucleotide having at least 80% sequence identity with SEQ ID NO: 42;
- d) an isolated polynucleotide having at least 90% sequence identity with SEQ ID NO: 42;
- e) an isolated polynucleotide having at least 95% sequence identity with SEQ ID NO: 42;
- f) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 42;
- g) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d), (e), or (f); and
- h) an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 42 and encodes an acyl CoA:cholesterol acyltransferase-like polypeptide.

21. An isolated nucleic acid sequence consisting essentially of a polynucleotide of the formula 5' X-(R<sub>1</sub>)<sub>n</sub>-(R<sub>2</sub>)<sub>n</sub>-(R<sub>3</sub>)<sub>n</sub>-Y 3', where X is hydrogen, Y is hydrogen or a metal R, and R<sub>1</sub> and R<sub>3</sub> are any nucleic acid, n is an integer between 0 and 3000, and R<sub>2</sub> is selected from the group consisting of:
- a) SEQ ID NO: 42 or degenerate variants thereof;
  - b) an isolated polynucleotide having at least 70% sequence identity to SEQ ID NO: 42;
  - c) an isolated polynucleotide having at least 80% sequence identity to SEQ ID NO: 42;
  - d) an isolated polynucleotide having at least 90% sequence identity to SEQ ID NO: 42;
  - e) an isolated polynucleotide having at least 95% sequence identity to SEQ ID NO: 42;
  - f) an isolated polynucleotide of at least 10 nucleic acids that hybridizes under stringent conditions to SEQ ID NO: 42;
  - g) an isolated polynucleotide complementary to a polynucleotide of (a), (b), (c), (d), (e), or (f); and

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- h) an isolated polynucleotide that hybridizes under stringent conditions to SEQ ID NO: 42 and encodes an acyl CoA:cholesterol acyltransferase-like polypeptide.
- 22. A recombinant nucleic acid construct comprising a regulatory sequence operably linked to polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide or a fragment thereof.
- 23. The recombinant nucleic acid construct of claim 22, wherein said lecithin:cholesterol acyltransferase-like polypeptide is a plant lecithin:cholesterol acyltransferase-like polypeptide.
- 24. A recombinant nucleic acid construct comprising a regulatory sequence operably linked to a polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide.
- 25. The recombinant nucleic acid construct of claim 24, wherein said acyl CoA:cholesterol acyltransferase-like polypeptide is a plant acyl CoA:cholesterol acyltransferase-like polypeptide.
- 26. The recombinant construct of claim 22, wherein said regulatory sequence comprises a heterologous regulatory sequence.
- 27. The recombinant construct of claim 24, wherein said regulatory sequence comprises a heterologous regulatory sequence.
- 28. The recombinant construct of claim 22, wherein said regulatory sequence is functional in a plant cell.
- 29. The recombinant construct of claim 24, wherein said regulatory sequence is functional in a plant cell.
- 30. The recombinant construct of claim 22, further comprising a termination sequence.

31. The recombinant construct of claim 24 further comprising a termination sequence.
32. The recombinant construct of claim 22 wherein said polynucleotide is selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 43, 44, 45, 46, 47, 48, 49, 50, 51, 73 and 75.
33. The recombinant construct of claim 24, wherein said polynucleotide is selected from the group consisting of SEQ ID NO: 33 and 42.
34. The recombinant construct of claim 22, wherein said regulatory sequence comprises a constitutive promoter.  
)
35. The recombinant construct of claim 24, wherein said regulatory sequence comprises a constitutive promoter.
36. The recombinant construct of claim 22, wherein said regulatory sequence comprises an inducible promoter.
37. The recombinant construct of claim 24, wherein said regulatory sequence comprises an inducible promoter.
38. The recombinant construct of claim 22, wherein said regulatory sequence is selected from the group consisting of a tissue specific promoter, a developmentally regulated promoter, an organelle specific promoter, and a seed specific promoter.  
)
39. The recombinant construct of claim 24, wherein said regulatory sequence is selected from the group consisting of a tissue specific promoter, a developmentally regulated promoter, an organelle specific promoter, and a seed specific promoter.
40. A host cell containing the recombinant construct of claim 22 or 24.

41. The host cell of claim 40, wherein said host cell is selected from the group consisting of plant cells, animal cells, insect cells, yeast, bacteria, bacteriophage and viruses.
42. The host cell of claim 40, wherein said host cell is a plant cell.
43. The host cell of claim 40, wherein said host cell expresses a lecithin:cholesterol acyltransferase-like polypeptide or an acyl CoA:cholesterol acyltransferase-like polypeptide.
44. The host cell of claim 43, wherein said cholesterol acyltransferase-like polypeptide is a plant acyltransferase-like polypeptide.
45. A plant comprising at least one host cell of claim 40.
46. The progeny of a plant of claim 45.
47. A seed from the plant of claim 45.
48. A plant comprising the recombinant construct of claim 22 or 24.
49. The progeny of a plant of claim 48.
50. A seed from the plant of claim 48.
51. A purified polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 3 with at least one conservative amino acid substitution, SEQ ID NO: 5, SEQ ID NO: 5 with at least one conservative amino acid substitution, SEQ ID NO: 7, SEQ ID NO: 7 with at least one conservative amino acid substitution, SEQ ID NO: 9, SEQ ID NO: 9 with at least one conservative amino acid substitution, SEQ ID NO: 74, SEQ ID NO: 74 with at least one conservative amino acid substitution, SEQ ID NO: 76 and SEQ ID NO: 76 with at least one conservative amino acid substitution.

52. A purified immunogenic polypeptide comprising at least 10 consecutive amino acids from an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 3 with at least one conservative amino acid substitution, SEQ ID NO: 5, SEQ ID NO: 5 with at least one conservative amino acid substitution, SEQ ID NO: 7, SEQ ID NO: 7 with at least one conservative amino acid substitution, SEQ ID NO: 9, SEQ ID NO: 9 with at least one conservative amino acid substitution, SEQ ID NO: 74, SEQ ID NO: 74 with at least one conservative amino acid substitution, SEQ ID NO: 76 and SEQ ID NO: 76 with at least one conservative amino acid substitution.
53. An antibody which specifically binds to an immunogenic polypeptide of claim 52.
- ) 54. A method for producing a lecithin:cholesterol acyltransferase-like polypeptide or an acyl CoA:cholesterol acyltransferase-like polypeptide comprising culturing a host cell of claim 40 under conditions permitting expression of said lecithin:cholesterol acyltransferase-like polypeptide or acyl CoA:cholesterol acyltransferase-like polypeptide.
55. The method of claim 54, further comprising isolating the cholesterol acyltransferase-like polypeptide from the host cell or from the medium in which the host cell is cultured.
- ) 56. A method for modifying the sterol content of a host cell, comprising transforming a host cell with a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide and culturing said host cell under conditions wherein said host cell expresses a lecithin:cholesterol acyltransferase-like polypeptide such that said host cell has a modified sterol composition as compared to host cells without the recombinant construct.
57. The method of claim 56, wherein said lecithin:cholesterol acyltransferase-like polypeptide is a plant lecithin:cholesterol acyltransferase-like polypeptide.

58. A method for modifying the sterol content of a host cell, comprising transforming a host cell with a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide and culturing said host cell under conditions wherein said host cell expresses an acyl CoA:cholesterol acyltransferase-like polypeptide such that said host cell has a modified sterol composition as compared to host cells without the recombinant construct.
59. The method of claim 58, wherein said acyl CoA:cholesterol acyltransferase-like polypeptide is a plant acyl CoA:cholesterol acyltransferase-like polypeptide.
60. The method of claim 56, wherein said modified sterol composition is an increase in sterol esters.
61. The method of claim 58, wherein said modified sterol composition is an increase in sterol esters.
62. The method of claim 56, wherein said polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide is selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 11, 73 and 75.
63. The method of claim 58 wherein said polynucleotide encoding a acyl CoA:cholesterol acyltransferase-like polypeptide is SEQ ID NO 33 OR 42.
64. The method of claim 56, wherein said regulatory sequence comprises a constitutive promoter.
65. The method of claim 58, wherein said regulatory sequence comprises a constitutive promoter.
66. The method of claim 56, wherein said regulatory sequence is an inducible promoter.
67. The method of claim 58, wherein said regulatory sequence is an inducible promoter.

68. The method of claim 56, wherein said regulatory sequence is a tissue specific promoter.
69. The method of claim 58, wherein said regulatory sequence is a tissue specific promoter.
70. The method of claim 56, wherein said regulatory sequence is a seed specific promoter.
71. The method of claim 58, wherein said regulatory sequence is a seed specific promoter.
- ) 72. The method of claim 56, wherein said polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide is in the antisense orientation.
73. The method of claim 58, wherein said polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide is in the antisense orientation.
74. The method of claim 72, wherein said modified sterol composition is a decrease in sterol esters.
75. The method of claim 73, wherein said modified sterol composition is a decrease in sterol esters.
- ) 76. A plant comprising a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide wherein expression of said recombinant construct results in modified sterol composition of said plant as compared to the same plant without said recombinant construct.  
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77. The plant of claim 76, wherein said lecithin:cholesterol acyltransferase-like polypeptide is a plant lecithin:cholesterol acyltransferase-like polypeptide.

78. The plant of claim 76, wherein said polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide is selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 11, 73 and 75.
79. A plant comprising a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide wherein expression of said recombinant construct results in modified sterol composition of said plant as compared to the same plant without said recombinant construct.  
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80. The plant of claim 79, wherein said acyl CoA:cholesterol acyltransferase-like polypeptide is a plant acyl CoA:cholesterol acyltransferase-like polypeptide.)
81. The plant of claim 79, wherein said polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide is SEQ ID NO: 33 or 42.
82. The plant of claim 76, wherein said regulatory sequence comprises a tissue specific promoter.
83. The plant of claim 79, wherein said regulatory sequence comprises a tissue specific promoter.
84. The plant of claim 76, wherein said regulatory sequence comprises a seed specific promoter.)
85. The plant of claim 79, wherein said regulatory sequence comprises a seed specific promoter.
86. The plant of claim 76, wherein said modified sterol composition is an increase in sterol esters.
87. The plant of claim 79, wherein said modified sterol composition is an increase in sterol esters.

88. The plant of claim 76, wherein the polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide is in the antisense orientation.
89. The plant of claim 79, wherein the polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide is in the antisense orientation.
90. An oil obtained from the plant of claim 76 or 79.
91. A method for producing an oil with a modified sterol composition comprising, providing a plant of claim 76 or 79 and extracting the oil from said plant.  
)
92. An oil produced by the method of claim 91.
93. A method for altering oil production by a host cell comprising, transforming a host cell with a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide and culturing said host cell under conditions wherein said host cell expresses a lecithin:cholesterol acyltransferase-like polypeptide such that said host cell has an altered oil production as compared to host cells without the recombinant construct.  
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94. The method of claim 93, wherein said lecithin:cholesterol acyltransferase-like polypeptide is a plant lecithin:cholesterol acyltransferase-like polypeptide.  
)
95. A method for altering oil production by a host cell comprising, transforming a host cell with a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide and culturing said host cell under conditions wherein said host cell expresses an acyl CoA:cholesterol acyltransferase-like polypeptide such that said host cell has an altered oil production as compared to host cells without the recombinant construct.  
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96. The method of claim 95, wherein said acyl CoA:cholesterol acyltransferase-like polypeptide is a plant acyl CoA:cholesterol acyltransferase-like polypeptide.

97. The method of claim 93, wherein said oil production is increased.
98. The method of claim 95, wherein said oil production is increased.
99. The method of claim 93, wherein said host cell is a plant cell.
100. The method of claim 95, wherein said host cell is a plant cell.
101. The method of claim 93, wherein said polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide is selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 11, 73 and 75. )
102. The method of claim 95, wherein said polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide is SEQ ID NO: 33 or 42.
103. The method of claim 93, wherein said regulatory sequence is a tissue specific promoter.
104. The method of claim 95, wherein said regulatory sequence is a tissue specific promoter.
105. The method of claim 93, wherein said regulatory sequence is a seed specific promoter. )
106. The method of claim 95, wherein said regulatory sequence is a seed specific promoter.
107. A plant comprising a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide wherein expression of said recombinant construct results in an altered production of oil by said plant as compared to the same plant without said recombinant construct.

108. The plant of claim 107, wherein said lecithin:cholesterol acyltransferase-like polypeptide is a plant lecithin:cholesterol acyltransferase-like polypeptide.
109. A plant comprising a recombinant construct containing a regulatory sequence operably linked to a polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide wherein expression of said recombinant construct results in an altered production of oil by said plant as compared to the same plant without said recombinant construct.  
5
110. The plant of claim 109, wherein said acyl CoA:cholesterol acyltransferase-like polypeptide is a plant acyl CoA:cholesterol acyltransferase-like polypeptide.  
)
111. The plant of claim 107, wherein said oil production is increased.  
)
112. The plant of claim 109, wherein said oil production is increased.  
)
113. The plant of claim 107, wherein said polynucleotide encoding a lecithin:cholesterol acyltransferase-like polypeptide is selected from the group consisting of SEQ ID NO: 2, 4, 6, 8, 10, 11, 73 and 75.  
)
114. The plant of claim 109, wherein said polynucleotide encoding an acyl CoA:cholesterol acyltransferase-like polypeptide is SEQ ID NO: 33 or 42.  
)
115. The plant of claim 107, wherein said regulatory sequence is a tissue specific promoter.  
)
116. The plant of claim 109, wherein said regulatory sequence is a tissue specific promoter.  
)
117. The plant of claim 107, wherein said regulatory sequence is a seed specific promoter.  
)
118. The plant of claim 109, wherein said regulatory sequence is a seed specific promoter.  
)
119. A food product comprising the oil of claim 90 or 92.

120. A food product comprising the plant of claim 107 or 109.

## SEQUENCE LISTING

<110> Monsanto Company

<120> PLANT STEROL ACYLTRANSFERASES

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<151> 1999-08-30

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35 Leu Ile Glu Ser Ile Asp Leu Phe Asn Asp Ala Leu Lys Asn Asn Glu  
290 295 300

Leu Ser Tyr Gly Gly Asn Lys Ile Ala Leu Pro Phe Asn Phe Ala Ile  
305 310 315 320



|    |             |              |             |             |             |             |      |
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|    | tgacacaata  | tggggcgggc   | ttgattggtc  | accggagaaa  | ggccacacct  | gttgtggaa   | 1560 |
|    | aaagcaaaag  | aacaacgaaa   | cttgggtgtga | agcagggtaa  | aacggagttt  | ccaagaaaaag | 1620 |
|    | tcctgttaac  | tatggaaagga  | tgatatcttt  | tgggaaagaa  | gtagcagagg  | ctgcgcacatc | 1680 |
|    | tgagattaat  | aatattgatt   | ttcgagtaag  | gacatataaa  | tcataataaa  | ccttgtacat  | 1740 |
| 5  | tttgtgattg  | tatgtaat     | atctgtacat  | tttatctgg   | gaagggtgct  | gtcaaaggc   | 1800 |
|    | agagtatccc  | aaatcacacc   | tgtcgtgacg  | tgtggacaga  | gtaccatgac  | atgggaattt  | 1860 |
|    | ctgggatcaa  | agctatcgct   | gagttataagg | tctacactgc  | tggtgaagct  | atagatctac  | 1920 |
|    | tacattatgt  | tgctcctaag   | atgatggcgc  | gtggtgcgc   | tcatttctct  | tatgggattt  | 1980 |
| 10 | ctgatgattt  | ggatgacacc   | aagttatcaag | atcccaataa  | ctggtcaaat  | ccgttagaga  | 2040 |
|    | caaagtaagt  | gatttcttga   | ttccaactgt  | atccctcgctc | ctgatgcatt  | atcagtcttt  | 2100 |
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|    | ctttctcaaa  | aaggcttgc    | cagttatatt  | gaggtgctaa  | agttgatata  | tgtgactt    | 2220 |
|    | gcttataaaat | cctccgttttgc | gttgggttctg | ctttttcaga  | ttaccgaatg  | ctcctgagat  | 2280 |
|    | ggaaaatctac | tcattatacgt  | gagttgggat  | accaacggaa  | cgagcatc    | tatacaagct  | 2340 |
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|    | cggaaatagc  | tgtctgaaag   | caggagttt   | caatgtggat  | ggggatgaaa  | cagtacctgt  | 2460 |
|    | cctaagtgcc  | gggtacatgt   | gtgc当地      | gtggcgtggc  | aagacaagat  | tcaacccttc  | 2520 |
|    | cggaaatcaag | acttacataa   | gagaatacaa  | tcactctccg  | ccggctaacc  | tgttggaaagg | 2580 |
|    | gcccgggacg  | cagagtgggt   | cccattgtga  | tatcatggga  | aactttgttt  | tgatcgaaga  | 2640 |
| 20 | tatcatgagg  | gttgcgcggcc  | gaggtaacgg  | gtctgtatata | ggacatgacc  | aggtccactc  | 2700 |
|    | tggcatattt  | gaatgggtcg   | agcgtattgt  | cctgaagctg  | tgaatatcat  | gatctcttta  | 2760 |
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| 25 | tgtttgaacg  | ataacgaaa    | acttgctcg   | ccatgtttgt  | ttgtcttgc   | aaaagcatca  | 3000 |
|    | atttgggtt   | tatacgtatg   | gtagaggatg  | attcaaaattt | gtgataaaatt | tggtatcaa   | 3060 |
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|       |    | aaaaaatgtta tagttctcat gaatactaac ataacaagtg aaagtaaatt taaaatgtt 180   |
| 40    |    | catggaccta acctggcgta acgttatgtc ttgccttca gcagaaagta aattactgac 240    |
|       |    | ggcttaggg acacctaaaa aggccccgtcc aatgttgacg acgatttga tgggtttggc 300    |
|       |    | acaccaaacct ggaccacccc caccgcctcc atcaggaaga ggtgtttcta cccatthaag 360  |
|       |    | gaagtgaagg aaatagatag ccccccattga atgcggaaacc accacaactt tcttaaaccc 420 |
|       |    | attggtgttca tacatttagct cgattttgtc cttagtctca cttaacgatt ggtcacgtac 480 |
| 45    |    | ctgctcggtt tcaatccaaa aactatagat tagtccaaag ctctacaaca atatgttaatt 540  |
|       |    | acatacacta aagttagctaa tcatggaggt cttagttagt atcattatca tcattctcta 600  |
|       |    | gaccaccagt gttgtcaatg tgatcatata ggttataata acgactaatac tgagcataacc 660 |
|       |    | tccgggttat ggaaagagag tctccaatca taangaggcc atgtgaaggt tcttgccttc 720   |
|       |    | atatccaaatt ttggccaaat tctctatgag aactgccaa gcaaagtgc atggtgccaa 780    |
| 50    |    | atagtctgca gccactagtc ctgggactgc tcggacacgg attcccggtg gatcgagacc 840   |
|       |    | ggtctcaactg tctagagata agtgctccaa ccagcacaat ggcctataaa tcaaattaca 900  |
|       |    | acaattaaac gaccaagtat acacttcaa ctaattcaga attgagaaaa tcgaaatgtc 960    |

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25 <220>  
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cacccctggt agattcatta caaaagcttg gctacgtga tggtgagact ctgtntggag 180  
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gatgctgtca ttgctatgca catgtggggc aagcaacctc gacccttta ttctaataacc 180  
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25 <222> (99)  
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<220>  
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35 <220>  
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<220>  
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attccacatt caatgggggt cttgtacttc ctacattta tgaaatgggt tgaagcacca 180  
gctccaatgg gtgggtgggg aggaccagat tggtgctcca aatatataaa ggcagttgt 240  
10 aacatttggtg gaccattttt aggtgttccc aaggctatag caggctatt ctcagctgag 300  
gccaaggata ttgctgttgc caggacgata gctccaggat tttanataaa cnatctgttt 360  
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agcaacaggt tgcaggaaac aatggatcgg ttggctgcna agttagaatc aanttataat 180  
25 gccgcagggn ggaagacaat aaacattata nttcattcta tggcggct tttccnnngan 240  
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cttttgggtga aatgnttcat gtgcctgcaa agcgatattt ttgagaaata tggtaagaat 180  
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tctttgggt aaatgtttca tgtgcctgca aagcgatatt tttgagaaat atgttaagaa 180  
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10 aaat 244

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<211> 263  
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<213> Glycine max

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ggaggattgg ttgcattgta ttttgcggca aacaaccctc atttagtgaa aggtgtcgca 180  
ttgcctaaggc aacacccccc tgggggtttc tgccaaatcc cataaaaagt ccaagactag 240  
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<211> 311  
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<222> (1)..(311)  
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35 acggacatcc t 311

<210> 20  
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<213> Zea mays

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gacttccgct acgcgcggc gtccccccgc cagacgtccg aggtgtactc ccgcctacttc 180  
aaggagctga tggagctggt cgaggccgc aacgagagga cccggaagaa ggccgtcatac 240  
45 ctcggccaca gttcggcgg catggtcgcg ctcgagttcg tccggAACAC tccggccggc 300

|    |             |             |             |             |             |             |      |
|----|-------------|-------------|-------------|-------------|-------------|-------------|------|
|    | tggccgcg    | agcacatcga  | gcgcctcg    | tcgtcg      | cgcgtccc    | cgccgggtc   | 360  |
|    | ctggagccgg  | tgcgcaactt  | cgcgtccggg  | acggacatcc  | tmtacgtgcc  | agcgacgacg  | 420  |
|    | ccgctggcca  | cgcgaggccat | gtgragragc  | ttcgagagcg  | ccatcgtaa   | cttcccgtcg  | 480  |
|    | ccggccgtgt  | tcggggcgt   | gcaggcgccg  | ctcggtgtca  | ccagggagcg  | gaactactcc  | 540  |
| 5  | gcgtccgcg   | acgacatgg   | gcgcgttc    | gcgcgcgtcg  | gtcccg      | ggccgcggag  | 600  |
|    | cccttcagga  | gacggggcgt  | ccccaaagatg | ggcagctcg   | cgggcggat   | ggtgcggcatg | 660  |
|    | acgtacatca  | gcgggggtcg  | caacaggacg  | ccgtcgccgc  | tggtgttctg  | gggcgacgac  | 720  |
|    | ttcgacgcgg  | ccccggaggt  | ggccgcgtac  | ggggacggag  | atggcaagat  | caatttgatc  | 780  |
|    | agcgtcttgg  | cgtttgagaa  | ggagatgcgt  | ccggcagccgg | agcagaagaa  | gcagttcaaa  | 840  |
| 10 | tccatcaaga  | tcgataaggc  | ccagcattct  | acgatcgta   | cggatgattt  | tgcctgcac   | 900  |
|    | agggtcattc  | aagaaaattgt | tgaggccaat  | aatcagaaga  | ttccatccta  | aattcttcat  | 960  |
|    | gtcatgtatg  | cattaccgag  | ctgtggggc   | caatagtgg   | ttgggagtgg  | ggacatcggt  | 1020 |
|    | tccgtgctta  | aaacggtcgt  | ggtgtggct   | caattcaatc  | gatttagttat | ttgttaacgt  | 1080 |
|    | caattgcttgc | cctcaaaaaaa | aaaaaaaaaa  | aaaaaaaaaa  | aaaaaaaaaa  | aaaaaaaaaa  | 1140 |
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<213> Zea mays

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30 <213> Zea mays

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atcgctgttc cgttatgatttgc gagaactgccc ccatcaatgc ttgaggagag agatctgtac 180
35 tttcacaaat taaagtttgt aacacttgcc tcaaacttgc tttatgaagcaac caatgctata 240
catctgttag gatcagaatggc agttaatggc ccattgacggta ttcaagggttcc tgctcacaa 300
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45 tatgatttcc gtcaaaagcaa caggctctca gagacacttg acagattttc taaaaagctg 180  
gagtcagtgt acacagctt cttggggaaaag aagatcaatc tcattactca ttcaatgggg 240  
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agttggatcg caattgctgc accattccar ggtccccctg ggtamataac taccaktytg 360  
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 atgcascaat tgctacttga gtgcccatca atctatgagk tgctgscaam ccctaactt 480  
 ccagtggaga gacatcccac tgctacagat ttggagagag aatttggata mcagtggcaa 540  
 5 gaaaagtgcc ctgttagagt cgtatgagc tgaggaagca ataaagatga ttaaagaggg 600  
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 15 gccaggcgag cttccctcta tggtcagcac actgaccacg agccggaaag gcaaggaggg 1200  
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 20 cctgcttgc aatgttcaga accgaaaaca caggccctgt tctttttttt cctttttaaa 1500  
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 <211> 227  
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 25 <213> Zea mays

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35 <210> 25  
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 <212> DNA  
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 <222> (1)..(1587)  
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 gacgaccacg ccctgtttgc catcgacattt cttgtatccctt cctggttgtt agaacttctc 180  
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 10 gacaatgtg aattaccaga tactgtgagc ttttacaata tatacggac atcttatgaa 900  
 actccatacg atgtatgcta tggctcagaa agctctccga ttggagattt gtcagaagtg 960  
 tgtcacacag tgccggcata cacttatgtg gatggagattt gcacgggtcc catagaatcg 1020  
 gcaacgggctg atgggttctc tggtcgaaagaa agagttggcg tcaaggcggc ccaccgtggc 1080  
 ctgctgtccg atgagaacgtt attcaagctt ctcagaataat ggctcggtgt gagcgagaag 1140  
 15 aagttagtgcgt ggcgttgcgt gtctaaatcc tactccaaag tgacctaattt ggggtgcctg 1200  
 tagttcttca ggaagactgt tattttggcc ttccctcctg aagagaagat gaaacaaaat 1260  
 tctgggtgattt gtattgtatg tctgcacgtat gtaaatctt gcaagctgca cggaacaagg 1320  
 gattagtgcc cttgtacgtat gatcatttgg caggcattt ttttgaacc tangggcata 1380  
 ttntttgtnc cttccactct ggacntagt aagaataatnt gaatcgacct tanttnnaan 1440  
 20 nnngtctgnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn 1500  
 nnnnnnnnnn nnaaaaaaaaa awgkgaagcc gntnnnnntt tnnaaagnnt tttnnnaaaa 1560  
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1587

<210> 26  
 <211> 300  
 25 <212> DNA  
 <213> Zea mays

<220>  
 <221> unsure  
 <222> (1)..(300)

30 <223> n=unknown

<400> 26  
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 atgaatcatg atgtgatgtt tcatgttttc tggcttttc ataaatatacc 180  
 35 atgtcaattt cccccatttt ctaggtatcc actangtatg tcaacaaatg gatttgcatt 240  
 gcttgcctt tccaaggtaa cttatggac atttcaattt tttattanat natgggncc 300

<210> 27  
 <211> 1240  
 <212> DNA  
 40 <213> Zea mays

<220>  
 <221> unsure  
 <222> (1)..(1240)  
 <223> n=unknown

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 tgccatattt gtaacacaga ttactatata caggttcgtg atcaaacttt gaggcagaata 120  
 aagascaata ttgaactcat agwagsgaca aatgggtggaa atagggtgggt ggkmgatccc 180

acnactccat ggggtcnnnn atttntgcn ttttacgnna tggntcgaag ccctcctccg 240  
 tgggggcagt gggtccgaac tggnntgaga accatataaa gctgtaatga atattggagg 300  
 atctttctta ggagttccta aggctgttc tgggtttt ttcttctaag caaaaagatgt 360  
 tgccgggtgc taggtataag taatgattca ttatataaa gaaaaaggga atagcaaaag 420  
 5 aatgaatatt attggatgct cgacaagctt gggagctt tgctccaaag ccatctctg 480  
 gacccaccaa gtccaggag tgctgcctc tgatcctcat catcaggaac aggctcaagt 540  
 atgcaccgac ggtaccgtga ggtcatttct atcctgtatgc aacaccatgt acttgttcat 600  
 ggcaaggtca ggactgacaa gacccatct gctgggtca tggatgtcat ttccatccct 660  
 aagacaaaacg agaactacag gctgcttcg tttcaccca atcagggatg agatgcca 720  
 10 gttcaagctc tacaagggtga ggtctgttca gtttggccag aaagacatcc cctatctgaa 780  
 cacctacgac gaccgcacca tccgctaccc cgacccgctc atcaaggcca acgacacat 840  
 caagatcgat ctggagacca acaagatcat ggacttcatc atgtttgacg tcggcaacgt 900  
 ggtcatggtg atcggcagga ggaataccgg gctgttagga gtatcaara taaggagaa 960  
 gataaagggc aacttcgaga ccatccacgt gctgcttgra gcttttgc atgtctagtt 1020  
 15 ttctcctatt tttgtacag gaaaacatag aatgaaattc aaatttggtg gccacaaaag 1080  
 tgtggagact tgatttcata taaagtttagg cttAACATTA gtgcaaacag ttgttattta 1140  
 gtttagattt agagtacact atgtatgcgt tgtttgacaa tgcttattta tgatatattg 1200  
 aatggtaattt atttatatta attaattaaa aaaaaaaaaa 1240

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 <210> 28  
 20 <211> 324  
 <212> DNA  
 <213> Zea mays

<400> 28  
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 25 catatgtcta taagttggcc ccacaggcag aatgttatat acctttccga attgacacct 120  
 cggctgaagg cggggaggaa aatagctgtct taaaaggggg tttttactta gccgatggtg 180  
 atgaaactgt tccagttctt agtgcggct acatgtgtc aaaaggatgg cgtggcaaaa 240  
 ctcgttcaa ccctgcggc agcaagactt acgtgagaga atacagccat tcaccaccc 300  
 ctactctcctt ggaaggcagg ggca 324

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 30 <210> 29  
 <211> 254  
 <212> DNA  
 <213> Zea mays

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 35 gaataaaagag caacattgaa ctcatggtag caacaatgg tggaaatagg gtgggtgtga 60  
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 ctcccattggg gggtggcggt ggtccagact ggtgtgagaa gcatattaaa gctgtaatga 180  
 atattggagg acctttctta ggagttccta aggctgttc tggccttttc tcattgtaa 240  
 ccaaagatgt tgcc 254

40 <210> 30  
 <211> 518  
 <212> DNA  
 <213> Mus musculus

<400> 30  
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 attcggcactc atgggcacga ggactccatc atgttctca agctttatcc ctaccggat 120  
 gtcaacctgt ggtgccgcca gcaagggtc aaggccaaag ctgtctctac agggaaag 180

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gtcagtgggg ctgctcgag caagctgtga gctatccaga caacctgacc taccgagatc 240
tcgattactt cattttgct cctactttgt gttatgaact caacccccc cggccccccc 300
gaatacgaga gcgccctctg ctacgacgag ttcttgagat gctctttttt acccagcttc 360
aagtggggct gatccaacag tggatggtcc ctactatcca gaactccatg gaagccctt 420
5 caagagcttc tgcagtttg gagaccgcga gttctacaga gattggtgga atgctgagtc 480
tgtcacccgac ttttggcaga actggaatat cccccgtgg 518

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<210> 31  
<211> 299  
<212> DNA  
10 <213> Mus musculus  
  
<400> 31  
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gcaatgcagc tgtgtgggtg acactcatca ttgggcaacc ggtggctgtc tcatgtatgt 120  
ccacgactac tacgtgtctca actacgatgc cccagtgggt catagactac tgccaaaggc 180  
15 agccctccct aacctggcc tggagttctg gaggggttcc tggctgcctg cacactcctc 240  
ctagtctqqq aqqcctctct qcccctatgc gctactcctg ctcttgggaa tggcatttg 299

<210> 32  
<211> 1895  
<212> DNA  
20 <213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Inferred cDNA sequence

<220>  
25 <221> unsure  
<222> (1)..(1895)  
<223> n=unknown

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tgggtgtga gtctcagagc ttggcatgga gaccagacag ggctgggtct gcaaggctga 120  
ggctgcgcgc ctgagctcg gctgggacgt gcccagaggt tttgggagga tctgggtga 180  
gtaccctgtg gccaggacta aaggggctnc accctcttgtt ccattccctcg cagatctga 240  
gcaatgccccg gttatttctg gagaacctca tcaagtatgg catcctggtg gaccccatcc 300  
agtggttgc tctgttctg aaggatccct atagctggcc cgccccatgc ctggttattg 360  
35 cgcccaatgt ctttgcgtg gctgcattcc aggttgagaa gcgcctggcg gtgggtgccc 420  
tgacggagca ggcgggactg ctgctgcacg tggccaaacctt ggcaccattt ctgtgttcc 480  
cagcggctgt ggtcttaactg gttgagtcta tcaactccagt gggctccctg ctggcgctga 540  
tggcgcacac catcctcttc ctaaagctct tctccttaccg cgacgtcaaac tcatggtgc 600  
40 gcaggggccag ggccaaggct gcctctgcag ggaagaaggc cagcagtgtt gtcggccgc 660  
acaccgttag ctacccggac aatctgaccc accgcgcattt ctactacttc ctttcgccc 720  
ccaccttgcg ctacgagctc aactttccccc gctctccccc catccggaaag cgctttctgc 780  
tgcgacggat ccttggatgt ctgttcttca cccagctcca ggtggggctg atccagaoagt 840  
ggatggtccc caccatccag aactccatga agcccttcaa ggacatggac tactcagc 900  
45 tcatcgagcg cctcctgaaag ctggcgggtcc ccaatcacctt catctggctc atcttcttct 960  
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atttcttaccg ggactgggtgg aactccgagt ctgtcaccta cttctggcag aactggaaaca 1080  
tccctgtgca caagtgggtgc atcagacact tctacaagcc catgcttcga cggggcagca 1140  
gcaagtggat ggccaggaca ggggtgttcc tggccctcgcc cttcttccac gagtacctgg 1200

tgagcgtccc tctgcgaatg ttccgcctct gggcgttcac gggcatgatg gctcagatcc 1260  
 cactggcctg gttcgtggc cgcttttcc agggcaacta tggcaacgca gctgtgtggc 1320  
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 tcaaactatga ggccccagcg gcagaggcct gagctgcacc tgagggcctg gcttctact 1440  
 5 gcccacccac acccgctgcc agagcccacc ttccttccta ggcctcgagt gctgggatg 1500  
 ggcttggctg cacagcatcc tcctctggc ccagggaggc ctctctgccc ctatgggct 1560  
 ctgtcctgca cccctcaggg atggcgacag caggccagac acagtctgtat gccagctggg 1620  
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 tcaagcctgccc aggggcctgg ggcctgggtgg ggggtatggc cacacccaca agggcgagtg 1740  
 10 ccagagctgt gtggacagct gtcccaggac ctgcgggaa gcagcagctc cactgcagca 1800  
 gggcgggcat ggccggtagg ggagtgcaa ggccaggcag acgcacccat tccccacact 1860  
 cccctaccta gaaaagctca gtcaggcgt cctct 1895

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 <210> 33  
 <211> 1766  
 15 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Inferred cDNA  
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20 <400> 33  
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 gctctcgcc tcggaggacc ggctcgccgg tttccatcca gggtgttagt gggccatgg 120  
 tagacgaaga ggaggtgcga gacgcgcgtg tgggccccga cttggcgcc ggggtgtacg 180  
 ctccggctcc ggctccgggtt cccgcctccag cccacaccccg ggacaaaagac cggcagacca 240  
 25 gcgtggcga cggccactgg gagctgagggt gccatcgctc gcaagactct ttgttcagct 300  
 cagacagcgg tttcagcaat taccgtggta tcctgaattt gtgcgtggtg atgctgatecc 360  
 tgagtaatgc aaggttattt ttagagaatc ttatcaagta tggcatctcg gtggatccca 420  
 tccaggtgtt gtctctgttt ctgaaggacc cttacagctg gctgccccca tgcttgatca 480  
 ttgcatccaa tatctttattt gtggctacat ttcagattga gaagcgcctg tcagtggtg 540  
 30 ccctgacaga gcagatgggg ctgctgctac atgtgtttaa cctggccaca attatctgt 600  
 tcccagcage tggccctta ctgggttagt ctatcactcc agtgggttcc ctgtttgctc 660  
 tggcatcata ctccatcata ttcccaaaatcc cccggatgtc aatctgtgg 720  
 gccgcgcagcg aagggtcaag gccaaagctg tgcgtgcagg gaagaaggc agtgggctg 780  
 ctgcccagaa cactgtaaatc tatccggaca acctgaccta ccgagatctc tattactca 840  
 35 tctttgtcc tactttgtgt tatgaactca actttctcg atccccccga atacgaaagc 900  
 gctttctgtc acggcggtt cttagatgc tcttttcac ccagcttcaa gtggggctga 960  
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 40 gagaccgcga gttctacagg gactgggttga atgctgagtc tgcacctac tttggcaga 1200  
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 tgggcagcaa caaatggatg gccaggactg gggctttt ggcgtcagcc ttcttcctatg 1320  
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 45 ctcaaggccccc actggcctgg attgtgaacc gcttcttca aggaaactat ggcaatgcag 1440  
 ctgtgtgggt gacactcatttggcacaac cggtggtgtt gtcatgtat gtccacgact 1500  
 actacgtgtc caactatgtt gccccagtg ggccctgagc tactgcacaaa ggccaggccct 1560  
 ccctaacctg ggccctggagt tctggggcctt cctgcacact cctctctatg 1620  
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 50 agctatctaa aaaaaaaaaaaaaaaa 1766

<210> 34  
<211> 409  
<212> PRT  
<213> Homo sapiens

5 <400> 34  
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Ile Tyr His Met Phe Ile Ala Leu Leu Ile Leu Phe Ile Leu Ser Thr  
20 25 30  
  
10 Leu Val Val Asp Tyr Ile Asp Glu Gly Arg Leu Val Leu Glu Phe Ser  
35 40 45  
  
Leu Leu Ser Tyr Ala Phe Gly Lys Phe Pro Thr Val Val Trp Thr Trp  
50 55 60  
  
15 Trp Ile Met Phe Leu Ser Thr Phe Ser Val Pro Tyr Phe Leu Phe Gln  
65 70 75 80  
  
His Trp Arg Thr Gly Tyr Ser Lys Ser Ser His Pro Leu Ile Arg Ser  
85 90 95  
  
Leu Phe His Gly Phe Leu Phe Met Ile Phe Gln Ile Gly Val Leu Gly  
100 105 110  
  
20 Phe Gly Pro Thr Tyr Val Val Leu Ala Tyr Thr Leu Pro Pro Ala Ser  
115 120 125  
  
Arg Phe Ile Ile Ile Phe Glu Gln Ile Arg Phe Val Met Lys Ala His  
130 135 140  
  
25 Ser Phe Val Arg Glu Asn Val Pro Arg Val Leu Asn Ser Ala Lys Glu  
145 150 155 160  
  
Lys Ser Ser Thr Val Pro Ile Pro Thr Val Asn Gln Tyr Leu Tyr Phe  
165 170 175  
  
Leu Phe Ala Pro Thr Leu Ile Tyr Arg Asp Ser Tyr Pro Arg Asn Pro  
180 185 190  
  
30 Thr Val Arg Trp Gly Tyr Val Ala Met Lys Phe Ala Gln Val Phe Gly  
195 200 205  
  
Cys Phe Phe Tyr Val Tyr Ile Phe Glu Arg Leu Cys Ala Pro Leu  
210 215 220  
  
35 Phe Arg Asn Ile Lys Gln Glu Pro Phe Ser Ala Arg Val Leu Val Leu  
225 230 235 240  
  
Cys Val Phe Asn Ser Ile Leu Pro Gly Val Leu Ile Leu Phe Leu Thr  
245 250 255  
  
Phe Phe Ala Phe Leu His Cys Trp Leu Asn Ala Phe Ala Glu Met Leu  
260 265 270

Arg Phe Gly Asp Arg Met Phe Tyr Lys Asp Trp Trp Asn Ser Thr Ser  
 275 280 285  
 Tyr Ser Asn Tyr Tyr Arg Thr Trp Asn Val Val Val His Asp Trp Leu  
 290 295 300  
 5 Tyr Tyr Tyr Ala Tyr Lys Asp Phe Leu Trp Phe Phe Ser Lys Arg Phe  
 305 310 315 320  
 Lys Ser Ala Ala Met Leu Ala Val Phe Ala Val Ser Ala Val Val His  
 325 330 335  
 10 Glu Tyr Ala Leu Ala Val Cys Leu Ser Phe Phe Tyr Pro Val Leu Phe  
 340 345 350  
 Val Leu Phe Met Phe Phe Gly Met Ala Phe Asn Phe Ile Val Asn Asp  
 355 360 365  
 Ser Arg Lys Lys Pro Ile Trp Asn Val Leu Met Trp Thr Ser Leu Phe  
 370 375 380  
 15 Leu Gly Asn Gly Val Leu Leu Cys Phe Tyr Ser Gln Glu Trp Tyr Ala  
 385 390 395 400  
 Arg Arg His Cys Pro Leu Lys Asn Pro  
 405  
 20 <210> 35  
 <211> 409  
 <212> PRT  
 <213> Mus musculus  
 <400> 35  
 25 Arg Gln Ser Leu Leu Asp Glu Leu Phe Glu Val Asp His Ile Arg Thr  
 1 5 10 15  
 Ile Tyr His Met Phe Ile Ala Leu Leu Ile Leu Phe Val Leu Ser Thr  
 20 25 30  
 Ile Val Val Asp Tyr Ile Asp Glu Gly Arg Leu Val Leu Glu Phe Asn  
 35 40 45  
 30 Leu Leu Ala Tyr Ala Phe Gly Lys Phe Pro Thr Val Ile Trp Thr Trp  
 50 55 60  
 Trp Ala Met Phe Leu Ser Thr Leu Ser Ile Pro Tyr Phe Leu Phe Gln  
 65 70 75 80  
 35 Pro Trp Ala His Gly Tyr Ser Lys Ser Ser His Pro Leu Ile Tyr Ser  
 85 90 95  
 Leu Val His Gly Leu Leu Phe Leu Val Phe Gln Leu Gly Val Leu Gly  
 100 105 110

Phe Val Pro Thr Tyr Val Val Leu Ala Tyr Thr Leu Pro Pro Ala Ser  
115 120 125

Arg Phe Ile Leu Ile Leu Glu Gln Ile Arg Leu Ile Met Lys Ala His  
130 135 140

5 Ser Phe Val Arg Glu Asn Ile Pro Arg Val Leu Asn Ala Ala Lys Glu  
145 150 155 160

Lys Ser Ser Lys Asp Pro Leu Pro Thr Val Asn Gln Tyr Leu Tyr Phe  
165 170 175

Leu Phe Ala Pro Thr Leu Ile Tyr Arg Asp Asn Tyr Pro Arg Thr Pro  
10 180 185 190

Thr Val Arg Trp Gly Tyr Val Ala Met Gln Phe Leu Gln Val Phe Gly  
195 200 205 )

Cys Leu Phe Tyr Val Tyr Ile Phe Glu Arg Leu Cys Ala Pro Leu  
210 215 220

15 Phe Arg Asn Ile Lys Gln Glu Pro Phe Ser Ala Arg Val Leu Val Leu  
225 230 235 240

Cys Val Phe Asn Ser Ile Leu Pro Gly Val Leu Ile Leu Phe Leu Ser  
245 250 255

Phe Phe Ala Phe Leu His Cys Trp Leu Asn Ala Phe Ala Glu Met Leu  
20 260 265 270

Arg Phe Gly Asp Arg Met Phe Tyr Lys Asp Trp Trp Asn Ser Thr Ser  
275 280 285 )

Tyr Ser Asn Tyr Tyr Arg Thr Trp Asn Val Val His Asp Trp Leu  
290 295 300 )

25 Tyr Tyr Tyr Val Tyr Lys Asp Leu Leu Trp Phe Phe Ser Lys Arg Phe  
305 310 315 320

Lys Ser Ala Ala Met Leu Ala Val Phe Ala Leu Ser Ala Val Val His  
325 330 335

Glu Tyr Ala Leu Ala Ile Cys Leu Ser Tyr Phe Tyr Pro Val Leu Phe  
30 340 345 350

Val Leu Phe Met Phe Phe Gly Met Ala Phe Asn Phe Ile Val Asn Asp  
355 360 365

Ser Arg Lys Arg Pro Ile Trp Asn Ile Met Val Trp Ala Ser Leu Phe  
370 375 380

35 Leu Gly Tyr Gly Leu Ile Leu Cys Phe Tyr Ser Gln Glu Trp Tyr Ala  
385 390 395 400 )

Arg Gln His Cys Pro Leu Lys Asn Pro  
405

<210> 36  
<211> 429  
<212> PRT  
<213> *Saccharomyces cerevisiae*

5 <400> 36

Asp Lys Ala Asp Ala Pro Pro Gly Glu Lys Leu Glu Ser Asn Phe Ser  
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Gly Ile Tyr Val Phe Ala Trp Met Phe Leu Gly Trp Ile Ala Ile Arg  
20 25 30

10 Cys Cys Thr Asp Tyr Tyr Ala Ser Tyr Gly Ser Ala Trp Asn Lys Leu  
35 40 45

) Glu Ile Val Gln Tyr Met Thr Thr Asp Leu Phe Thr Ile Ala Met Leu  
50 55 60

15 Asp Leu Ala Met Phe Leu Cys Thr Phe Phe Val Val Phe Val His Trp  
65 70 75 80

Leu Val Lys Lys Arg Ile Ile Asn Trp Lys Trp Thr Gly Phe Val Ala  
85 90 95

Val Ser Ile Phe Glu Leu Ala Phe Ile Pro Val Thr Phe Pro Ile Tyr  
100 105 110

20 Val Tyr Tyr Phe Asp Phe Asn Trp Val Thr Arg Ile Phe Leu Phe Leu  
115 120 125

His Ser Val Val Phe Val Met Lys Ser His Ser Phe Ala Phe Tyr Asn  
130 135 140

) 25 Gly Tyr Leu Trp Asp Ile Lys Gln Glu Leu Glu Tyr Ser Ser Lys Gln  
145 150 155 160

Leu Gln Lys Tyr Lys Glu Ser Leu Ser Pro Glu Thr Arg Glu Ile Leu  
165 170 175

Gln Lys Ser Cys Asp Phe Cys Leu Phe Glu Leu Asn Tyr Gln Thr Lys  
180 185 190

30 Asp Asn Asp Phe Pro Asn Asn Ile Ser Cys Ser Asn Phe Phe Met Phe  
195 200 205

Cys Leu Phe Pro Val Leu Val Tyr Gln Ile Asn Tyr Pro Arg Thr Ser  
210 215 220

35 Arg Ile Arg Trp Arg Tyr Val Leu Glu Lys Val Cys Ala Ile Ile Gly  
225 230 235 240

Thr Ile Phe Leu Met Met Val Thr Ala Gln Phe Phe Met His Pro Val  
245 250 255

Ala Met Arg Cys Ile Gln Phe His Asn Thr Pro Thr Phe Gly Gly Trp  
260 265 270

Ile Pro Ala Thr Gln Glu Trp Phe His Leu Leu Phe Asp Met Ile Pro  
 275                    280                    285

Gly Phe Thr Val Leu Tyr Met Leu Thr Phe Tyr Met Ile Trp Asp Ala  
 290                    295                    300

5   Leu Leu Asn Cys Val Ala Glu Leu Thr Arg Phe Ala Asp Arg Tyr Phe  
 305                    310                    315                    320

Tyr Gly Asp Trp Trp Asn Cys Val Ser Phe Glu Glu Phe Ser Arg Ile  
 325                    330                    335

10   Trp Asn Val Pro Val His Lys Phe Leu Leu Arg His Val Tyr His Ser  
 340                    345                    350

Ser Met Gly Ala Leu His Leu Ser Lys Ser Gln Ala Thr Leu Phe Thr  
 355                    360                    365 )

Phe Phe Leu Ser Ala Val Phe His Glu Met Ala Met Phe Ala Ile Phe  
 370                    375                    380

15   Arg Arg Val Arg Gly Tyr Leu Phe Met Phe Gln Leu Ser Gln Phe Val  
 385                    390                    395                    400

Trp Thr Ala Leu Ser Asn Thr Lys Phe Leu Arg Ala Arg Pro Gln Leu  
 405                    410                    415

Ser Asn Val Val Phe Ser Phe Gly Val Cys Ser Gly Pro  
 20                    420                    425

<210> 37  
<211> 432  
<212> PRT  
<213> Saccharomyces cerevisiae

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Gly Leu Tyr Val Ala Phe Trp Met Ala Ile Ala Phe Gly Ala Val Lys  
 20                    25                        30

30   Ala Leu Ile Asp Tyr Tyr Tyr Gln His Asn Gly Ser Phe Lys Asp Ser  
 35                    40                        45

Glu Ile Leu Lys Phe Met Thr Thr Asn Leu Phe Thr Val Ala Ser Val  
 50                    55                        60

Asp Leu Leu Met Tyr Leu Ser Thr Tyr Phe Val Val Gly Ile Gln Tyr  
 35   65                    70                    75                    80

Leu Cys Lys Trp Gly Val Leu Lys Trp Gly Thr Thr Gly Trp Ile Phe  
 85                    90                        95

Thr Ser Ile Tyr Glu Phe Leu Phe Val Ile Phe Tyr Met Tyr Leu Thr  
100 105 110

Glu Asn Ile Leu Lys Leu His Trp Leu Ser Lys Ile Phe Leu Phe Leu  
115 120 125

5 His Ser Leu Val Leu Leu Met Lys Met His Ser Phe Ala Phe Tyr Asn  
130 135 140

Gly Tyr Leu Trp Gly Ile Lys Glu Glu Leu Gln Phe Ser Lys Ser Ala  
145 150 155 160

Leu Ala Lys Tyr Lys Asp Ser Ile Asn Asp Pro Lys Val Ile Gly Ala  
10 165 170 175

Leu Glu Lys Ser Cys Glu Phe Cys Ser Phe Glu Leu Ser Ser Gln Ser  
180 185 190

Leu Ser Asp Gln Thr Gln Lys Phe Pro Asn Asn Ile Ser Ala Lys Ser  
195 200 205

15 Phe Phe Trp Phe Thr Met Phe Pro Thr Leu Ile Tyr Gln Ile Glu Tyr  
210 215 220

Pro Arg Thr Lys Glu Ile Arg Trp Ser Tyr Val Leu Glu Lys Ile Cys  
225 230 235 240

Ala Ile Phe Gly Thr Ile Phe Leu Met Met Ile Asp Ala Gln Ile Leu  
20 245 250 255

Met Tyr Pro Val Ala Met Arg Ala Leu Ala Val Arg Asn Ser Glu Trp  
260 265 270

Thr Gly Ile Leu Asp Arg Leu Leu Lys Trp Val Gly Leu Leu Val Asp  
275 280 285

)

25 Ile Val Pro Gly Phe Ile Val Met Tyr Ile Leu Asp Phe Tyr Leu Ile  
290 295 300

Trp Asp Ala Ile Leu Asn Cys Val Ala Glu Leu Thr Arg Phe Gly Asp  
305 310 315 320

Arg Tyr Phe Tyr Gly Asp Trp Trp Asn Cys Val Ser Trp Ala Asp Phe  
30 325 330 335

Ser Arg Ile Trp Asn Ile Pro Val His Lys Phe Leu Leu Arg His Val  
340 345 350

Tyr His Ser Ser Met Ser Ser Phe Lys Leu Asn Lys Ser Gln Ala Thr  
355 360 365

35 Leu Met Thr Phe Phe Leu Ser Ser Val Val His Glu Leu Ala Met Tyr  
370 375 380

Val Ile Phe Lys Lys Leu Arg Phe Tyr Leu Phe Phe Phe Gln Met Leu  
385 390 395 400

Gln Met Pro Leu Val Ala Leu Thr Asn Thr Lys Phe Met Arg Asn Arg  
405 410 415

Thr Ile Ile Gly Asn Val Ile Phe Trp Leu Gly Ile Cys Met Gly Pro  
420 425 430

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<210> 38  
<211> 1942  
<212> DNA  
<213> *Arabidopsis thaliana*

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| 20 | atgctacgtt tacgtatcga ccgtcggttc cagctcatcg gagggcgaga gagagtccac 600<br>ttagctccga cgcaatcttc aaacagagcc atgcccgtt attcaaccttc tttgttagtag 660<br>ttcttattgc tggtaaacagt agactcatca tcgaaaatct tatgaagtat ggttggttga 720<br>tcagaacggaa ttctgtgtt agtcaagat cgctgcgaga ttggccgctt ttcatgtgtt 780<br>gtatatccctt ttcatgttcc ccttggctg cctttacgtt tgagaaattt gtacttcaga 840                |   |
| 25 | aatacatatc agaacctgtt gtcatcttc ttcatattat tateaccatg acagagggtt 900<br>tgtatccagt ttacgtcacc ctaaggtgtt attctgcattt tttatcaggt gtcactttga 960<br>tgctcctcac ttgcattgtt tggctaaagt tggtttctta tgctcataact agctatgaca 1020<br>taagatccctt agccaatcga gctgataagg ccaatcttgc agtctccttac tacgttagct 1080<br>tgaagagctt ggcataatttc atggtcgctc ccacattgtt ttatcagcca agttatccac 1140          |   |
| 30 | gttctgcattt tatacggaaag ggttgggtgg ctcgtcaatt tgcaaaactg gtcatattca 1200<br>ccggattcat gggatttata atagaacaat atataaatcc tattgtcagg aactcaaaagc 1260<br>atcctttgaa aggcatctt ctatatgcta ttgaaagagt gttgaagctt tcagttccaa 1320<br>atttatatgt gtggctctgc atgttctact gtttcttcca cctttggta aacatattgg 1380<br>cagagcttctt ctgcttcggg gatcgtgaat tctacaaaga ttgggtggaaat gcaaaaagtg 1440        |   |
| 35 | tgggagatta ctggagaatg tggaaatatgc ctgttcataa atggatgggtt cgacatatat 1500<br>acttcccggtt cttgcgcagc aagataccaa agacactcgc cattatcatt gctttccctag 1560<br>tctctgcagt ctttcatgag ctatgcattcg cagttcccttgc tggctcttcc aagctatggg 1620<br>cttttcttgg gattatgtt caggtgcctt tggcttccat cacaaaactat ctacaggaaaa 1680<br>ggtttggctc aacgggtgggg aacatgtatct tctggttcat cttctgcattt ttcggacaac 1740 |   |
| 40 | cgtatgtgtt gtttctttat taccacgacc tgatgaaccg aaaaggatcg atgtcatgaa 1800<br>acaactgttc aaaaaatgac ttcttc当地 catctatggc ctcgttggat ctccggttgc 1860<br>gttgggtgg ttctgtatgtt aaaaacgacaa atagtgttat aaccattgaa gaagaaaaaaga 1920<br>caatttagagt tgggtatcg ca 1942  |   |

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<211> 520  
<212> PRT  
<213> *Arabidopsis thaliana*

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Gly Gly Glu Phe Val Asp Leu Asp Arg Leu Arg Arg Arg Lys Ser Arg  
5 20 25 30

Ser Asp Ser Ser Asn Gly Leu Leu Ser Gly Ser Asp Asn Asn Ser  
35 40 45

Pro Ser Asp Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp  
50 55 60

10 Ser Val Val Asn Asp Asp Ala Gln Gly Thr Ala Asn Leu Ala Gly Asp  
65 70 75 80

) Asn Asn Gly Gly Asp Asn Asn Gly Gly Arg Gly Gly Glu  
85 90 95

Gly Arg Gly Asn Ala Asp Ala Thr Phe Thr Tyr Arg Pro Ser Val Pro  
15 100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe  
115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile  
130 135 140

20 Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp  
145 150 155 160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp  
165 170 175

) Pro Leu Phe Met Cys Cys Ile Ser Leu Ser Ile Phe Pro Leu Ala Ala  
25 180 185 190

Phe Thr Val Glu Lys Leu Val Leu Gln Lys Tyr Ile Ser Glu Pro Val  
195 200 205

Val Ile Phe Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro  
210 215 220

30 Val Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr  
225 230 235 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala  
245 250 255

His Thr Ser Tyr Asp Ile Arg Ser Leu Ala Asn Ala Ala Asp Lys Ala  
35 260 265 270

Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe  
275 280 285

Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Ala  
290 295 300

Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile  
305 310 315 320

5 Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile  
325 330 335

Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile  
340 345 350

Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys  
10 355 360 365

Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu  
370 375 380 )

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys  
385 390 395 400

15 Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp  
405 410 415

Met Val Arg His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys  
420 425 430

Thr Leu Ala Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu  
20 435 440 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Leu  
450 455 460

Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln  
465 470 475 480 )

25 Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe  
485 490 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu  
500 505 510

Met Asn Arg Lys Gly Ser Met Ser  
30 515 520

&lt;210&gt; 40

&lt;211&gt; 29

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
oligonucleotide primer

&lt;400&gt; 40

5 tgcaaattga cgagcacacc aacccttc

29

&lt;210&gt; 41

&lt;211&gt; 28

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

10 &lt;220&gt;

<223> Description of Artificial Sequence: Synthetic  
oligonucleotide primer

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aaggatgctt tgagttcctg acaatagg

28

15 &lt;210&gt; 42

&lt;211&gt; 1942

&lt;212&gt; DNA

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 42

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tccgctcttt ccctctccat tagattctgt ttctctttt aatttcttct gcatgcttct 180  
cgattctctc tgacgcctct ttctcccgaa cgctgtttcg tcaaaccgtt ttcgaaatgg 240  
cgattttggaa ttctgctggc gttaactacgg tgacggagaa cggggcggga gagttcgctg 300  
25 atcttgatag gtttcgtcga cggaaatcga gatcggttcc ttctaaccggaa cttcttctct 360  
ctgggtccga taataattct ccttcggatg atgttggagc tcccgccgac gttaggatc 420  
ggattgattc cgttgttaac gatgacgcgtc agggAACAGC caatttggcc ggagataata 480  
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30 tttagctccga cgcaatcttcc aaacagagcc atgcccggatt attcaacccat tttgttagtag 660  
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tcagaacggaa tttctgggtt agttcaagat cgctgcgaga ttggccgctt ttcatgtgtt 780  
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35 tttatccatg ttacgtcacc ctaagggtgtt attctgttcc ttatccatg gtcactttga 960  
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40 cccgattcat gggatttata atagaacaat atataaaatcc tattgtcagg aactcaaagc 1260  
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atttatatgt gtggctctgc atgttctact gcttcttcca cttttggta aacatattgg 1380  
cagagcttctc ctgcttcggg gatcgtaat tctacaaaga ttgttggaaat gcaaaaaatgt 1440  
tgggagatta ctggagaatg tggaaatatgc ctgttcataa atgatgggtt cgacatatat 1500  
45 acttccccgtt cttgcgcagc aagataccaa agacactcgc cattatcatt gctttccat 1560  
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cttttcttgg gattatgttt caggtgcctt tggcttcat cacaaactat ctacaggaaa 1680  
ggttggctc aacgggtggg aacatgatct tctggttcat cttctgcattt tcggacaac 1740

cgatgtgtgt gcttctttat taccacgacc tcatgtgaaccg aaaaggatcg atgtcatgaa 1800  
acaactgttc aaaaaatgac ttcttcataa catctatggc ctgcgttggat ctccgttgat 1860  
gttgtggat ttctgtatgct aaaacgacaa atagtgttat aaccattgaa gaagaaaaga 1920  
caatttagagt tggttatcg ca 1942

- 5 <210> 43  
<211> 234  
<212> DNA  
<213> Glycine max
- 10 <220>  
<221> unsure  
<222> (1)..(234)  
<223> n=unknown
- 15 <400> 43  
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ctatcctcgc acacctata ttccaaaggg ttggctgtt cgccaacttg tcaactgata 120  
atatttacag gagttatggg atttataata gaacaataca ttaatcccat tgtacaaaat 180  
tcacagcatc ctctcaaggg aaaccttctt tacgcccattc agagagttct gaag 234
- 20 <210> 44  
<211> 267  
<212> DNA  
<213> Glycine max
- 25 <400> 44  
ctgctttgt atctgggtgc acgttgatgc tattaacttg cattgtgtgg ttaaaaattgg 60  
tgtcatatgc acatacaaac tatgatatga gagcacttac tgtttcaaat gaaaagggag 120  
aaacatttacc caatactttg atatggatca tccgtacact gtgaccttca ggagtttgc 180  
atacttcatg gttgcttca cattatgtca tcagacaagc tatactcgca caccttcaat 240  
tcgaaagggt tgggtgttgc gtcaact 267
- 30 <210> 45  
<211> 275  
<212> DNA  
<213> Glycine max
- 35 <220>  
<221> unsure  
<222> (1)..(275)  
<223> n=unknown
- 40 <400> 45  
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gatgatccgc cacctatatt ttccatgttt aaggcacggc ataccaaagg ccgttgcct 120  
tttaattgccc ttccctgggtc tgctttatcc catgagctgt gcatcgctgt tccttgccca 180  
catattcaag tngtgggttt cnngnngaatt nagtttcagg tnccttgggt ttcnaccnna 240  
attnntnggc naaaaaattc cnngaacccc ggggg 275

<210> 46  
<211> 257  
<212> DNA  
<213> Glycine max

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ggctttggaa aaaactggca tgcttccttc aacaagtggc ttgtgaggta tatatacatt 120  
cctcttgggg gatctaagaa aaagctacta aatgtgtggg ttgtttcac atttgttgc 180  
atctggcatg atttagatg gaagcttctt tcatggcat gggtgacgtg tttattcttc 240  
10 atccctgagt tggttt 257

)<210> 47  
<211> 253  
<212> DNA  
<213> Zea mays

15 <400> 47  
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cgaaaatggta tatcaaagga agttgctgtt ttatatacgt tcttgttctt gctgtaccc 120  
atgagttatg tggctgtt ccctgccaca tactcaagtt ctgggctttt tttaggaatc 180  
atgcttcaga tccccatcat catattgaca tcataacctca aaaataaatt cagtgacaca 240  
20 atggttggca ata 253

<210> 48  
<211> 254  
<212> DNA  
<213> Zea mays

25 <400> 48  
tgaagtatgg cttattaata agatctggct tttggtttaa tgctacatca ttgcgagact 60  
ggccactgtc aatgtgttgc cttagtcac ccatattcc ccttggtgca ttgcagtcg 120  
aaaagttggc attcaacaat ctctttagtg atccctgtac tacctgtttt cacatccccc 180  
ttacaacatt tggaaattgtta tatccagtgc tcgtgattct taagtgtgat tctgcagttt 240  
30 ttcaggctt tgtg 254

<210> 49  
<211> 262  
<212> DNA  
<213> Zea mays

35 <400> 49  
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gccactgtca atgtgttgc ttgttaccatattccc cttggtgcat ttgcagtcg 120  
aaagttggca ttcaacaatc tcatttagtga tcctgtactt acctgtttt acatccccc 180  
tacaacattt gaaattgtat atccagtgtc cgtgattttt aagtgtgattt ctgcagttt 240  
40 acaggcttgc ttgttgc 262

<210> 50  
<211> 325  
<212> DNA  
<213> Zea mays

<220>  
<221> unsure  
<222> (1)..(325)  
<223> n=unknown

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cacatantca natctnggc a tnnchngggat catngttcag ataccgntgg nattcttgc 120  
aagatatctc catgctacgt tcaagcatgt aatggtgggc aacatgatan tttggntcn 180  
cagtatagtc ggacagccga tgtnnnnnna tctatactac catgacgtca tgaacaggca 240  
ggcccaggca agtagatagt ncggcagaga catgtacttc aacatcganc atcagnagca 300  
nacngagcga gcggcangaa ncagc 325

<210> 51  
<211> 519  
<212> DNA  
15 <213> Mortierrella alpina )  
  
<220>  
<221> unsure  
<222> (1)..(519)  
<223> n=unknown  
  
20 <400> 51  
gagnnnnngna acgttagcc tnccgtagcc gccaaaatcc aagggnncnac cnaccctncg 60  
ttanactnaa ttngaaaatn cnncnccaaac ttnaggnact tnnagncccc cnacttgac 120  
aacggagcac tatatttacc ccgtggtngt tcaaccccagc catctcaccc ttgcgagcat 180  
tgtgctgtct cttgatacccc ttcatgctta actatctcat gatctttac atcatttgc 240  
25 agtgcacatcg caacgcctt gggaaactaa gttgctttgc ggatcgcaac ttttacgagg 300  
atgggtggaa ctgcgtcage tttgatgagt gggcacgcaa atgaaacaag cctgtgcaac 360  
acttcttgct ccgccacgtg tacgactcga gcatccgagt ccttccactt gtccgaaatc 420  
caatgcccn aattgcaaac gtccctccc ggtcgtaat gcttcaacg aacctgggtg 480  
aagaatgggt ggtgacaacg ttaaagtgcg cccggatc 519 )  
  
30 <210> 52  
<211> 45  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
35 <223> Description of Artificial Sequence:  
Oligonucleotide primer  
  
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ggatccgcgg ccgcacaatg aaaaaaataat cttcacatta ttcg 45  
  
<210> 53  
40 <211> 40  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide primer

5 <400> 53  
ggatccccctg caggtcattc attgacggca ttaacattgg 40

<210> 54  
<211> 44  
<212> DNA  
<213> Artificial Sequence

10 <220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide primer  
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<400> 54  
ggatccgcgg ccgcacaatg ggagcgaatt cgaaatcagt aacg 44

15 <210> 55  
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<212> DNA  
<213> Artificial Sequence

<220>  
20 <223> Description of Artificial Sequence: Synthetic  
oligonucleotide primer

<400> 55  
ggatccccctg caggttaata cccactttta tcaagctccc 40

<210> 56  
25 <211> 41  
<212> DNA  
<213> Artificial Sequence

<220>  
30 <223> Description of Artificial Sequence: Synthetic  
oligonucleotide primer

<400> 56  
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<210> 57  
<211> 41  
35 <212> DNA  
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<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide primer

<400> 57  
ggatccctg cagttatgc atcaacagag acacttacag c 41

<210> 58  
<211> 41  
5 <212> DNA  
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<220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide primer

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<210> 59  
<211> 38  
<212> DNA  
15 <213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide primer

<400> 59  
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<210> 60  
<211> 39  
<212> DNA  
<213> Artificial Sequence )

25 <220>  
<223> Description of Artificial Sequence:  
Oligonucleotide primer

<400> 60  
tcgacctgca ggaagcttag aaatggcgat tttggattc 39 )

30 <210> 61  
<211> 36  
<212> DNA  
<213> Artificial Sequence )

<220>  
35 <223> Description of Artificial Sequence:  
Oligonucleotide primer )

<400> 61  
ggatccgcgg ccgcctcatga catcgatcct ttccgg 36 )

<210> 62  
<211> 56  
<212> DNA  
<213> Artificial Sequence

5 <220>  
<223> Description of Artificial Sequence: Annealed  
oligonucleotide adapter

<400> 62  
cgcgatttaa atggcgcgcc ctgcaggcgg ccgcctgcag ggccgcgccat ttaaat 56

10 <210> 63  
<211> 32  
<212> DNA  
<213> Artificial Sequence

)  
15 <220>  
<223> Description of Artificial Sequence: Ligating  
oligonucleotide

<400> 63  
tcgaggatcc gccccgcaa gcttcctgca gg 32

20 <210> 64  
<211> 32  
<212> DNA  
<213> Artificial Sequence

25 <220>  
<223> Description of Artificial Sequence: Ligating  
oligonucleotide

<400> 64  
tcgacctgca ggaagttgc ggccgcggat cc 32

30 <210> 65  
<211> 32  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Ligating  
oligonucleotide

35 <400> 65  
tcgacctgca ggaagttgc ggccgcggat cc 32

<210> 66  
<211> 32  
<212> DNA  
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5 <220>  
<223> Description of Artificial Sequence: Ligating  
oligonucleotide

<400> 66  
tcgaggatcc gcggccgcaa gcttcctgca gg

32

10 <210> 67  
<211> 36  
<212> DNA  
<213> Artificial Sequence

15 <220>  
<223> Description of Artificial Sequence: Ligating  
oligonucleotide

<400> 67  
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36

20 <210> 68  
<211> 28  
<212> DNA  
<213> Artificial Sequence

25 <220>  
<223> Description of Artificial Sequence: Ligating  
oligonucleotide

<400> 68  
cctgcaggaa gcttgccggcc gcggatcc

28

30 <210> 69  
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<212> DNA  
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<220>  
<223> Description of Artificial Sequence: Ligating  
oligonucleotide

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36

<210> 70  
<211> 28  
<212> DNA  
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5 <220>  
<223> Description of Artificial Sequence: Ligating  
oligonucleotide

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28

10 <210> 71  
<211> 39  
<212> DNA  
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)<220>  
15 <223> Description of Artificial Sequence: Ligating  
oligonucleotide

<400> 71  
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39

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<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
25 <223> Description of Artificial Sequence: Ligating  
oligonucleotide

<400> 72  
ttggatccgc ggccgcaagc ttccctgcagg t

31

30 <210> 73  
<211> 2013  
<212> DNA  
<213> Arabidopsis thaliana

<400> 73  
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35 aaatcgacg gaggaggaa gtggtcgtgc atcgattttt gtttgtgggtt cattgggtgt 180  
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aatgaaactg ggttggatcc agctggattt agagttcgag ctgtatcagg actcgtggct 540  
gctgactact ttgctctgg ctactttgtc tggcagtgc tgattgctaa cttgcacat 600  
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aacacagagg tacgtgatca gactcttagc cgtataaaa gtaatataga gttgatggtt 720  
 tctaccaacg gtggaaaaaa agcagttata gttccgcatt ccatgggggt cttgtatTTT 780  
 ctacattta tgaagtgggt tgagggacca gtcctctgg gtggggggg tggccagat 840  
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 5 aaagctgtg cagggcttt ctctgctgaa gcaaaggatg ttgcagttgc cagagecatt 960  
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 atgacacgca catgggactc aacaatgtct atgttacca agggaggtga cacgatatgg 1080  
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 acgcagagtg gtgcctatgt tgatatcatg ggaaactttt cttgatcgaa agatatcatg 1920  
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 tttgaatggt cggagcgtat tgacctgaa ctg 2013

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 30 1 5 10 15

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Ser | Glu | Glu | Val | Val | His | Asp | Glu | Asp | Ser | Gln | Lys | Lys | Pro | His |
|     |     |     |     | 20  |     |     |     | 25  |     |     |     | 30  |     |     |     |

Glu Ser Ser Lys Ser His His Lys Lys Ser Asn Gly Gly Gly Lys Trp  
 35 35 40 45

35 Ser Cys Ile Asp Ser Cys Cys Trp Phe Ile Gly Cys Val Cys Val Thr  
 50 55 60

Trp Trp Phe Leu Leu Phe Leu Tyr Asn Ala Met Pro Ala Ser Phe Pro  
 65 70 75 80

Gln Tyr Val Thr Glu Arg Ile Thr Gly Pro Leu Pro Asp Pro Pro Gly  
 40 85 90 95

Val Lys Leu Lys Lys Glu Gly Leu Lys Ala Lys His Pro Val Val Phe  
 100 105 110

Ile Pro Gly Ile Val Thr Gly Gly Leu Glu Leu Trp Glu Gly Lys Gln  
 115 120 125

Cys Ala Asp Gly Leu Phe Arg Lys Arg Leu Trp Gly Gly Thr Phe Gly  
130 135 140

Glu Val Tyr Lys Arg Pro Leu Cys Trp Val Glu His Met Ser Leu Asp  
145 150 155 160

5 Asn Glu Thr Gly Leu Asp Pro Ala Gly Ile Arg Val Arg Ala Val Ser  
165 170 175

Gly Leu Val Ala Ala Asp Tyr Phe Ala Pro Gly Tyr Phe Val Trp Ala  
180 185 190

Val Leu Ile Ala Asn Leu Ala His Ile Gly Tyr Glu Glu Lys Asn Met  
10 195 200 205

) Tyr Met Ala Ala Tyr Asp Trp Arg Leu Ser Phe Gln Asn Thr Glu Val  
210 215 220

Arg Asp Gln Thr Leu Ser Arg Met Lys Ser Asn Ile Glu Leu Met Val  
225 230 235 240

15 Ser Thr Asn Gly Gly Lys Lys Ala Val Ile Val Pro His Ser Met Gly  
245 250 255

Val Leu Tyr Phe Leu His Phe Met Lys Trp Val Glu Ala Pro Ala Pro  
260 265 270

Leu Gly Gly Gly Gly Pro Asp Trp Cys Ala Lys Tyr Ile Lys Ala  
20 275 280 285

Val Met Asn Ile Gly Gly Pro Phe Leu Gly Val Pro Lys Ala Val Ala  
290 295 300

Gly Leu Phe Ser Ala Glu Ala Lys Asp Val Ala Val Ala Arg Ala Ile  
305 310 315 320

25 Ala Pro Gly Phe Leu Asp Thr Asp Ile Phe Arg Leu Gln Thr Leu Gln  
325 330 335

His Val Met Arg Met Thr Arg Thr Trp Asp Ser Thr Met Ser Met Leu  
340 345 350

Pro Lys Gly Gly Asp Thr Ile Trp Gly Gly Leu Asp Trp Ser Pro Glu  
30 355 360 365

Lys Gly His Thr Cys Cys Gly Lys Lys Gln Lys Asn Asn Glu Thr Cys  
370 375 380

Gly Glu Ala Gly Glu Asn Gly Val Ser Lys Lys Ser Pro Val Asn Tyr  
385 390 395 400

35 Gly Arg Met Ile Ser Phe Gly Lys Glu Val Ala Glu Ala Ala Pro Ser  
405 410 415

Glu Ile Asn Asn Ile Asp Phe Arg Gly Ala Val Lys Gly Gln Ser Ile  
420 425 430

Pro Asn His Thr Cys Arg Asp Val Trp Thr Glu Tyr His Asp Met Gly  
 435 440 445  
 Ile Ala Gly Ile Lys Ala Ile Ala Glu Tyr Lys Val Tyr Thr Ala Gly  
 450 455 460  
 5 Glu Ala Ile Asp Leu Leu His Tyr Val Ala Pro Lys Met Met Ala Arg  
 465 470 475 480  
 Gly Ala Ala His Phe Ser Tyr Gly Ile Ala Asp Asp Leu Asp Asp Thr  
 485 490 495  
 Lys Tyr Gln Asp Pro Lys Tyr Trp Ser Asn Pro Leu Glu Thr Lys Leu  
 10 500 505 510  
 Pro Asn Ala Pro Glu Met Glu Ile Tyr Ser Leu Tyr Gly Val Gly Ile  
 515 520 525  
 Pro Thr Glu Arg Ala Tyr Val Tyr Lys Leu Asn Gln Ser Pro Asp Ser  
 530 535 540  
 15 Cys Ile Pro Phe Gln Ile Phe Thr Ser Ala His Glu Glu Asp Glu Asp  
 545 550 555 560  
 Ser Cys Leu Lys Ala Gly Val Tyr Asn Val Asp Gly Asp Glu Thr Val  
 565 570 575  
 Pro Val Leu Ser Ala Gly Tyr Met Cys Ala Lys Ala Trp Arg Gly Lys  
 20 580 585 590  
 Thr Arg Phe Asn Pro Ser Gly Ile Lys Thr Tyr Ile Arg Glu Tyr Asn  
 595 600 605  
 His Ser Pro Pro Ala Asn Leu Leu Glu Gly Arg Gly Thr Gln Ser Gly  
 610 615 620  
 25 Ala His Val Asp Ile Met Gly Asn Phe Ala Leu Ile Glu Asp Ile Met  
 625 630 635 640  
 Arg Val Ala Ala Gly Gly Asn Gly Ser Asp Ile Gly His Asp Gln Val  
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 ttaggccata agagaagaag ggttattagt ggcagtgcaa aaagaaatga gcgtggcaaa 180  
 gatttcgaca ggaaaagaga cgggaacggt agaaaacgtt ggagagattc cagaagactg 240

|    |  |      |
|----|--|------|
|    | attttcattc ttgggtgcatt cttaggtgta cttttgccgt ttagcttgg cgcttatcat    | 300  |
|    | gttcataata gcgatagcga ctgtttgac aactttgtaa atttgattc acttaaaagtg     | 360  |
|    | tatttggatg attggaaaga tgttctcca caaggatataa gttcggttat tgatgatatt    | 420  |
|    | caggctggta actactccac atcttctta gatgatctca gtgaaaattt tgccgttgt      | 480  |
| 5  | aaacaactct tacgtgatta taatatcgag gccaaacatc ctgttgtaat ggttccttgt    | 540  |
|    | gtcatttcta cgggaattga aagctggga gttattggag acgtgagtg cgatagttct      | 600  |
|    | gcmcattttc gtaaacggct gtggggaaagt ttttacatgc tgagaacaat ggttatggat   | 660  |
|    | aaagtttgtt ggttgaaaaca tgtaatgtta gatcctgaaa caggtctgga cccaccgaac   | 720  |
| 10 | tttacgctac gtgcagcaca gggcttcgaa tcaactgatt atttcatcgc agggtatttg    | 780  |
|    | atttggaaaca aagtttcca aaatctggga gtaattggct atgaacccaa taaaatgacg    | 840  |
|    | agtgcgtcgt atgattggag gcttcataat ttagatctag aaagacgcga taggtacttt    | 900  |
|    | acgaagctaa aggaacaaat cgaactgttt catcaattga gtggtaaaaa agtttgttta    | 960  |
|    | attggacatt ctatgggttc tcagattatc ttttacttta tgaatgggt cgaggctgaa     | 1020 |
|    | ggccctctt acggtaatgg tggcgtggc tgggttaacg aacacataga ttcatcatt       | 1080 |
| 15 | aatgcagcag ggacgcttct gggcgctcca aaggcagttc cagctcta at tagtggtaa    | 1140 |
|    | atgaaagata ccattcaatt aaatacgtt a cccatgtat gttggaaaaa gttcttctca    | 1200 |
|    | agaattgaga gagaaaaat gttacaaacg tgggggtgta taccatcaat gctaccaaag     | 1260 |
|    | ggagaagagg tcattttggg ggatatgaag tcatcttcag aggatgcatt gaataacaac    | 1320 |
|    | actgacacat acggcaattt cattcgattt gaaaggaata cgagcgatgc tttcaacaaa    | 1380 |
| 20 | aatttgacaa taaaagacgc cattaacatg acattatcga tatcacctga atggctccaa    | 1440 |
|    | agaagaggtac atgagcagta ctcgttcggc tattccaaga atgaagaaga gttaaaaaa    | 1500 |
|    | aatgagctac accacaagca ctggtcgaat ccaatggaaag taccacttcc agaagctccc   | 1560 |
|    | cacatgaaaa tctattgtat atacggggtg aacaacccaa ctgaaagggc atatgtatat    | 1620 |
|    | aaggaagagg atgactcctc tgctctgaat ttgaccatcg actacgaaag caagcaacct    | 1680 |
| 25 | gtattcctca ccgaggggga cggAACGTT cccgtcggtt cgcattcaat gtgtcacaaa     | 1740 |
|    | tggccccagg gtgcattcacc gtacaaccct gcccgaatatta acgttactat tttggaaatg | 1800 |
|    | aaacaccaggc cagatcgatt tgatatacgt ggtggagcaa aaagegccga acacgtagac   | 1860 |
|    | atccctcggtca ggcggagtt gaacgattac atcttggaaa ttgcaagcgg taatggcgat   | 1920 |
|    | ctcgctcgagc cacgccaatt gtctaattt agccagtggg tttctcgat gccccttccca    | 1980 |
| 30 | atgtaa   | 1986 |

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<213> *Saccharomyces cerevisiae*

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Lys Arg Asp Gly Asn Gly Arg Lys Arg Trp Arg Asp Ser Arg Arg Leu  
 45 65 70 75 80

Ile Phe Ile Leu Gly Ala Phe Leu Gly Val Leu Leu Pro Phe Ser Phe  
85 90 95

Gly Ala Tyr His Val His Asn Ser Asp Ser Asp Leu Phe Asp Asn Phe  
 100 105 110

Val Asn Phe Asp Ser Leu Lys Val Tyr Leu Asp Asp Trp Lys Asp Val  
 115 120 125

5 Leu Pro Gln Gly Ile Ser Ser Phe Ile Asp Asp Ile Gln Ala Gly Asn  
 130 135 140

Tyr Ser Thr Ser Ser Leu Asp Asp Leu Ser Glu Asn Phe Ala Val Gly  
 145 150 155 160

10 Lys Gln Leu Leu Arg Asp Tyr Asn Ile Glu Ala Lys His Pro Val Val  
 165 170 175

Met Val Pro Gly Val Ile Ser Thr Gly Ile Glu Ser Trp Gly Val Ile  
 180 185 190

Gly Asp Asp Glu Cys Asp Ser Ser Ala His Phe Arg Lys Arg Leu Trp  
 195 200 205

15 Gly Ser Phe Tyr Met Leu Arg Thr Met Val Met Asp Lys Val Cys Trp  
 210 215 220

Leu Lys His Val Met Leu Asp Pro Glu Thr Gly Leu Asp Pro Pro Asn  
 225 230 235 240

20 Phe Thr Leu Arg Ala Ala Gln Gly Phe Glu Ser Thr Asp Tyr Phe Ile  
 245 250 255

Ala Gly Tyr Trp Ile Trp Asn Lys Val Phe Gln Asn Leu Gly Val Ile  
 260 265 270

Gly Tyr Glu Pro Asn Lys Met Thr Ser Ala Ala Tyr Asp Trp Arg Leu  
 275 280 285

25 Ala Tyr Leu Asp Leu Glu Arg Arg Asp Arg Tyr Phe Thr Lys Leu Lys  
 290 295 300

Glu Gln Ile Glu Leu Phe His Gln Leu Ser Gly Glu Lys Val Cys Leu  
 305 310 315 320

30 Ile Gly His Ser Met Gly Ser Gln Ile Ile Phe Tyr Phe Met Lys Trp  
 325 330 335

Val Glu Ala Glu Gly Pro Leu Tyr Gly Asn Gly Gly Arg Gly Trp Val  
 340 345 350

Asn Glu His Ile Asp Ser Phe Ile Asn Ala Ala Gly Thr Leu Leu Gly  
 355 360 365

35 Ala Pro Lys Ala Val Pro Ala Leu Ile Ser Gly Glu Met Lys Asp Thr  
 370 375 380

Ile Gln Leu Asn Thr Leu Ala Met Tyr Gly Leu Glu Lys Phe Phe Ser  
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<210> 77  
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<212> DNA  
<213> Artificial Sequence

5 <220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide primer

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10 <210> 78  
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<212> DNA  
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<400> 78  
ggatccccctg caggtcacag cttaggtca atacg

35

20 <210> 79  
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<212> DNA  
<213> Artificial Sequence

25 <220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide primer

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37

30 <210> 80  
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<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide primer

35 <400> 80  
ggatccccctg caggttacat tggcacact gtttcgaag

39

1/7

FIG. 1A

## ClustalW Formatted Alignments

Yeast(YN008W) M G T L F R R N V Q N Q K S D S D E N N K G G S V H N K R E S R N H I H H Q Q G L G H K R R G I S  
 Human LCAT  
 Rat LCAT  
 Af LCAT1  
 Af LCAT2  
 Af LCAT3  
 Af LCAT4

FIG. 1B

**Yeast (YNR008W)** D D L S E N F A V G K Q I L R D Y N I E A K H P - - - - - V V M V P G V I S T G L E S W G V I G D D  
**Human LCAT** - - - - - G N Q I E A K L - - D K P D V - - - - - V N W M C - - Y R K T E D F F T I W L D  
**Rat LCAT** - - - - - G N R I E A K L - - D K P N V - - - - - V N W L C - - Y R K T E D F F T I W L D  
**AtLCAT1** - - - - - G N Q I E V R L - - D R E Y K P S S V W C S S W L Y P I H K S G G W F R L V W L D  
**AtLCAT2** - - - - - G N Q I E V R L - - D C P Y - - - - - T P - - - - L D F N P L D L V W L D  
**AtLCAT3** - - - - - S T Q I E A W S I L D H E - - - - N - - - - G N E E R - - V W V R  
**AtLCAT4** - - - - - G S I I L N A V - - D H E - - - - S - - - - K S E I R - - V W V R  
  
**Yeast (YNR008W)** E C D S S A H F R K R L W G S F Y M L R T M X M D K V C W L K H V M L D P E T G L D P P N - F T L R  
**Human LCAT** - L N M F I P L G V D C W I D N T R V - - V Y N R S S G - - L V S N A P G V Q I R V P G - F G K T  
**Rat LCAT** - F N M F I P L G V D C W I D N T R V - - V Y N R S S G - - H M S N A P G V Q I R V P G - F G K T  
**AtLCAT1** - A A V L S P F T R G F S D R M M L - - Y D P D L D - - D Y Q N A P G V Q T R M P H - F G S T  
**AtLCAT2** - T T K E L S A V N C W F K - - C M - - V L D P Y N - - Q T D H P E C K S R P D - S G L  
**AtLCAT3** - F G A D H E F R T K M W S - - R F D P S T G K - - T I S L D P K T S I V V P Q D R A G L  
**AtLCAT4** - F L A N T A F K Q S L W S - - L Y N E P K T G Y - - T E P L D D N I E V L V E D D D H G L  
  
**Yeast (YNR008W)** A Q G F E S T D Y F I A G - - - - - Y W I W N K V E Q N L E G V I G Y E P N - K M T S A A Y D W R I I  
**Human LCAT** - S V E Y I D S S K L A G Y - - - - - L H T L V Q N L V N N G Y V R D E T V R A A P Y D W R I I  
**Rat LCAT** - Y S V E Y I D D N K L A G Y - - - - - L N T L V Q N L V N N G Y V R D E T V R A A P Y D W R I I  
**AtLCAT1** - K S L L Y I D P R L R D A T - - - - S Y M E H V K A L E K K C Y V N D Q I L G A P Y D F R Y  
**AtLCAT2** - S A I T E I D P G Y I T G P - - - - L S T V W K E W L K W C V E F G I E A N - A I V A V I V D W R I I  
**AtLCAT3** - H A I D V I D P D M I V G R - - E S V Y Y F H E M I V E M I G W G F E E G K G L F G F G Y D F R Q  
**AtLCAT4** - Y A I D I L D P S W F V K L C H L T E V Y H F H D M I E M L W G C G Y K K G T T L F G Y G Y D F R Q  
  
**Yeast (YNR008W)** A . . L D P . . G . . . . . V P . . . . . F . . . . .  
**Human LCAT** - . . . . . A P Y D W R L

FIG. 1C

**Yeast (YNR008W)**

|            |   |                   |   |                                 |                                   |     |
|------------|---|-------------------|---|---------------------------------|-----------------------------------|-----|
| A Y        | - - -                                   | L D L E R R D E R | R F T K E Q I E L F H Q   | L S C                           | E K V C L I G H S M G S O I I F Y | 340 |
| Human LCAT | E P                                     | - - -             | G Q E E R Y R K L A G L V E M H A A Y C                                     | K P V F G H S L C               | C I H L V C I H L V L H           | 350 |
| Rat LCAT   | A P                                     | - - -             | R Q Q D E Y Q K L A G L V E M Y A A Y C                                     | K P V F G H S L G               | C I H L V C I H L V L H           |     |
| Al LCAT1   | G L A A S G H P S R V A S Q F L Q D L K | -                 | V E K T S S E N E G K P V I L S H S M G G L E N                             | G E S I V F A H S M G G L L N   | K C I                             |     |
| Al LCAT2   | S P                                     | - - -             | T K L I E R D L I F H K L K L T F E T A L K L R C                           | G E S I V F A H S M G G L L N   | K C I                             |     |
| Al LCAT3   | S                                       | - - -             | N R L Q E T L D Q F A K K I E T V Y K A S G E K K I N V I S H S M G G L L N | G E S I V F A H S M G G L L N   | K C I                             |     |
| Al LCAT4   | S                                       | - - -             | N R I D L L I G U R K K I E T A Y K R S G G R K Y                           | G E S I V F A H S M G G L L N   | K C I                             |     |
|            |   | D . Y .           | K L K . E   | G K P V L I . H S M G . L . V F |                                   |     |

**Yeast (YNR008W)**

|                                   |  |                           |  |
|-----------------------------------|--|---------------------------|--|
| M K W V E A E G P L Y G N G C R G | W V N E H D S E T I N A A G T L L G A P K A V P A L I S C E M K D            | 400                       |  |
| M L R - - -                       | [Q P Q A W K D R F I D G E I S L G A P W G G S I K P M R I V L A S G D N - - |                           |  |
| M L R - - -                       | [Q P Q S W K D H F I D G E I S L G A P W G G S I K P M R I V L A S G D N - - |                           |  |
| M L R - - -                       | T T P S W R R K Y H K H E V A L A A E W G G T I S Q M K T F A S G N T - -    |                           |  |
| M L E W L R L E I A P K - - -     | H Y L K W L D Q H H A Y F A V G A P L L G S V E A I K S T L L S C V T F G    |                           |  |
| M G L - - -                       | H S D I F E K - - -  | A P F R G - - -           |  |
| M Y L - - -                       | H P E A F S K - - -  | A P G Y I T S T L L N G M |  |
| L                                 | H P E A F S K - - -  | A P G C I N D S I L T G V |  |

**Yeast (YNR008W)**

|   |   |   |   |
|---|---|---|---|
| T I Q L N T L A M Y G L E K F F S R I E R V K - - - | M L Q T W G G I P S M L P K G - - - E E V I X G D M | 450   |   |
| Human LCAT  | [Q G I P I M S S I K L K E - - -                    | E Q R I T T S P - - -                                     | X M F -   |
| Rat LCAT  | [Q G I P I M S N I K L R E - - -                    | E Q R I T T S P - - -                                     | X M F -   |
| Al LCAT1  | I G V P L Y N P L L V R R - - -                     | H Q R T S E S N Q - - -                                   | X L L -   |
| Al LCAT2  | L P V S - - -                                       | E G T A R L L S N S F A S S L W L M P F S K N C K G - - - | D N T S X T H F                                     |
| Al LCAT3  | S - F V - - -                                       | N G W E Q N F F V S K W S - - -                           | M H Q L I E C P S I Y E L M C C P Y F K W E L P     |
| Al LCAT4  | Q - F V - - -                                       | E G L E S F F F V S R W T - - -                           | M H Q L L V E C P S I Y E M M A N P D F K W K K Q W |

FIG. 2

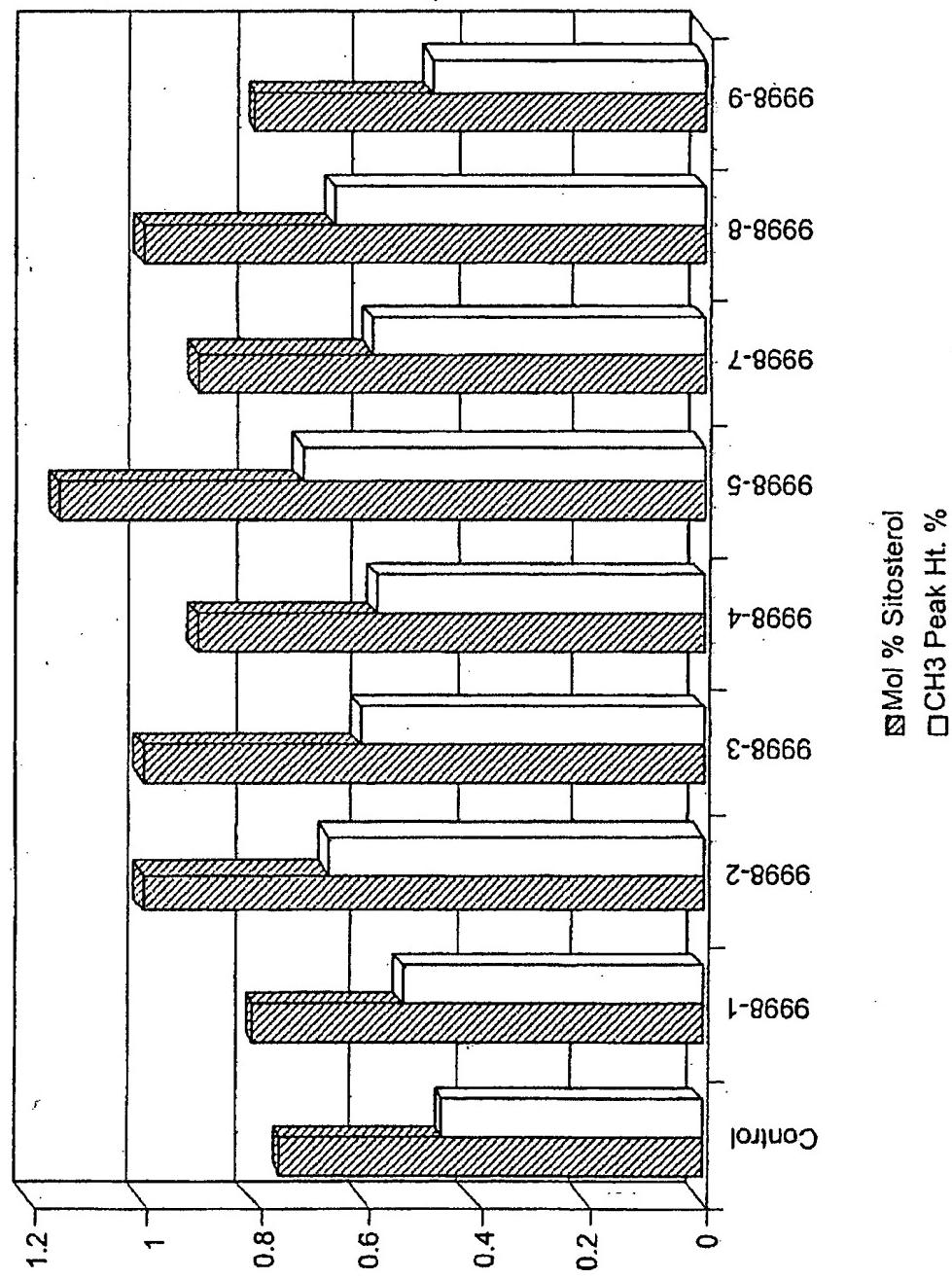
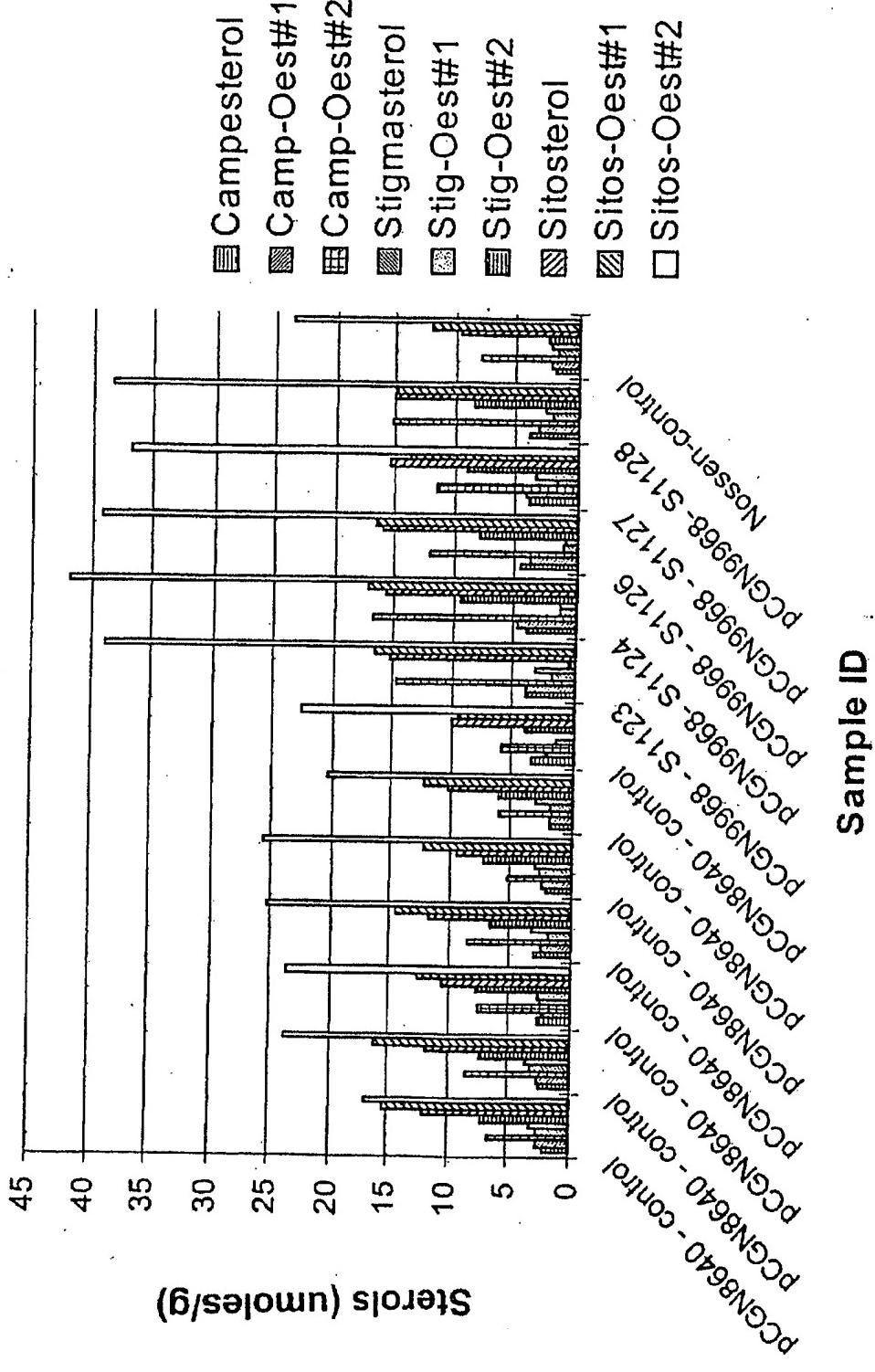


FIG. 3

## Sterol Distribution



**FIG. 4**

Sterol Distribution

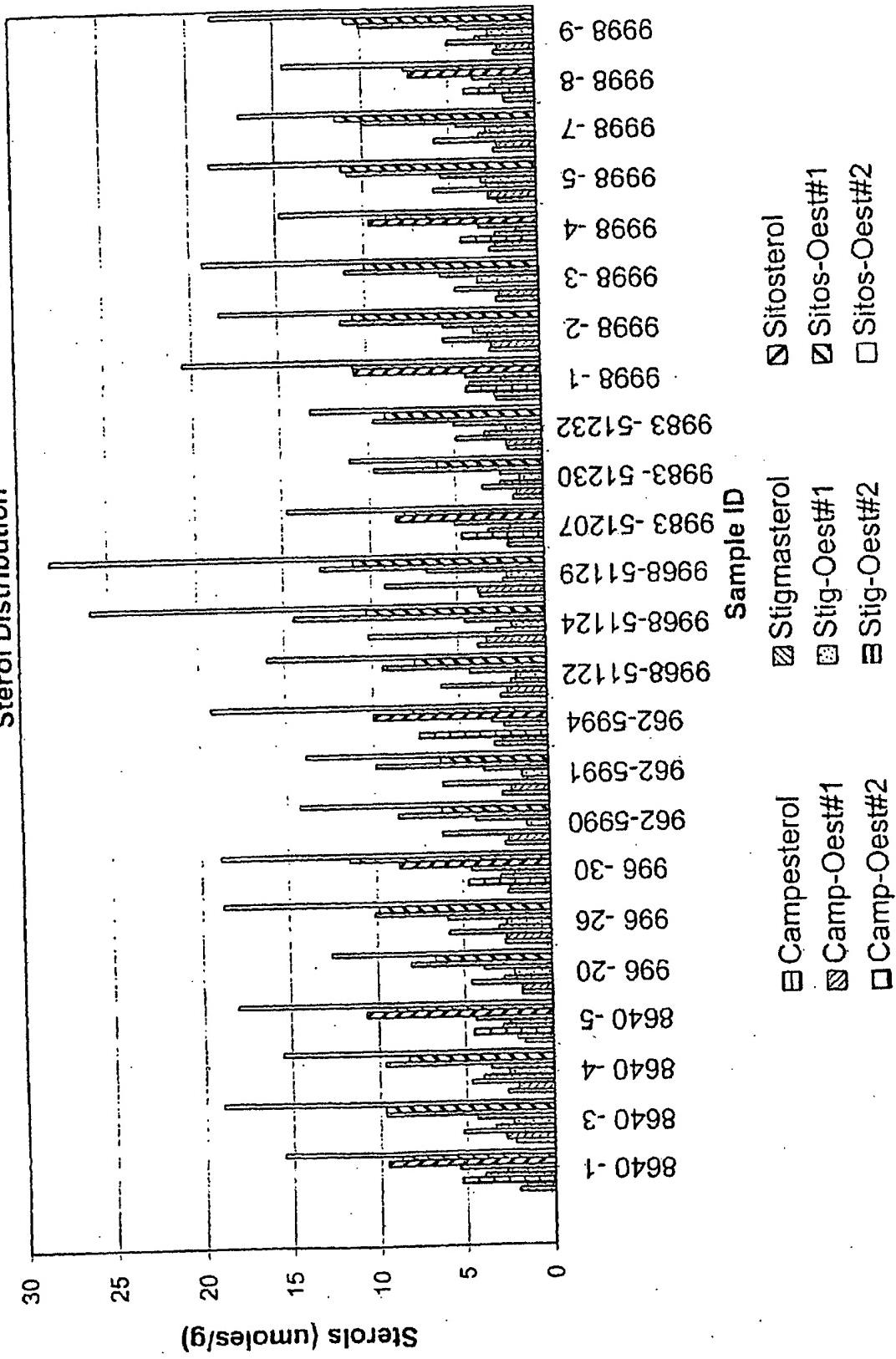
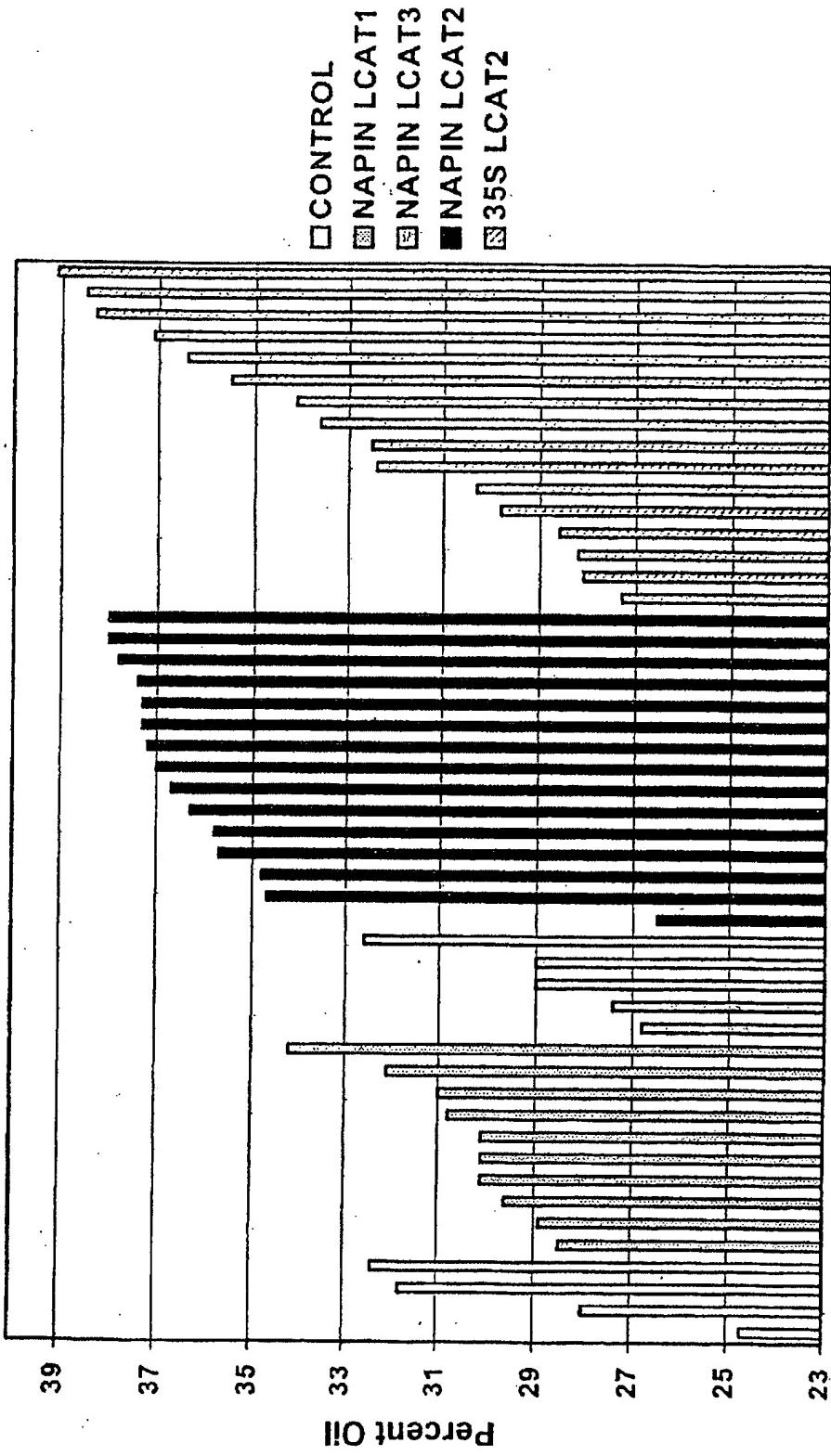


FIG. 5  
NIR Analysis of LCAT



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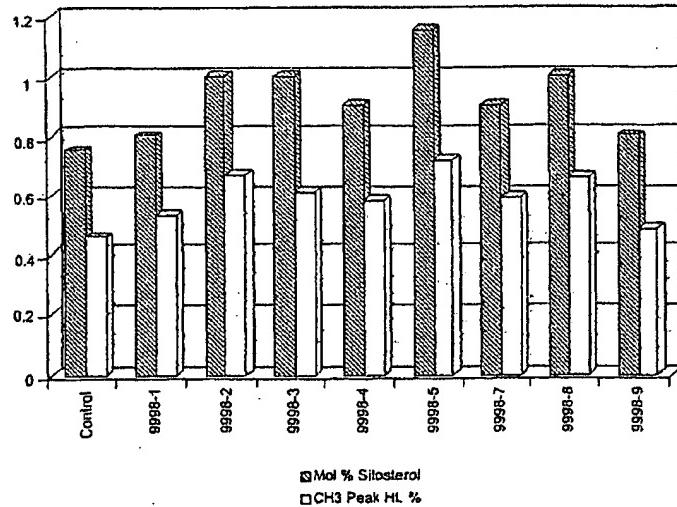
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[Continued on next page]

(54) Title: PLANT STEROL ACYLTRANSFERASES



(57) Abstract: The present invention is directed to lecithin: cholesterol acyltransferase-like polypeptides (LCAT) and acyl CoA: cholesterol acyltransferases-like polypeptides (ACAT). The invention provides polynucleotides encoding such cholesterol: acyltransferases-like polypeptides, polypeptides encoded by such polynucleotides, and the use of such polynucleotides to alter sterol composition and oil production in plants and host cells. Also provided are oils produced by the plants and host cells containing the polynucleotides and food products, nutritional supplements, and pharmaceutical composition containing plants or oils of the present invention. The polynucleotides of the present invention include those derived from plant sources.

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/23863

**A. CLASSIFICATION OF SUBJECT MATTER**  
**IPC 7 C12N15/82**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

**IPC 7 C12N**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.   |
|----------|--|---|
| X        | <p>HOBBS D H ET AL: "Cloning of a cDNA encoding diacylglycerol acyltransferase from <i>Arabidopsis thaliana</i> and its functional expression"<br/> <i>FEBS LETTERS, NL, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM,</i><br/> <i>vol. 452, no. 3, 11 June 1999 (1999-06-11), pages 145-149,</i><br/> <i>XP002122747</i><br/> <i>ISSN: 0014-5793</i><br/> <i>&amp; DATABASE EMBL [Online]</i><br/> <i>EBI</i><br/> <i>accession no. AJ131831.1,</i><br/> <i>10 June 1999 (1999-06-10)</i><br/> <i>see sequence</i><br/> <i>---</i><br/> <i>-/-</i> </p> | <p>1-4, 6, 7,<br/> 20-31,<br/> 33-50,<br/> 54-61,<br/> 63-77,<br/> 79-100,<br/> 102-112,<br/> 114-120</p> |

Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search

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Date of mailing of the international search report

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## INTERNATIONAL SEARCH REPORT

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| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |   |  |
|--|---|--|
| Category   | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.  |
| X  | DATABASE EMBL [Online]<br>EBI<br>accession no. AF164434,<br>26 July 1999 (1999-07-26)<br>NYKIFORUK C.L. ET AL: "Brassica napus<br>putative diacylglycerol acyltransferase<br>(DGAT1) mRNA"<br>XP002161573<br>see sequence<br>---  | 1-4,6,7,<br>20-31,<br>33-50,<br>54-61,<br>63-77,<br>79-100,<br>102-112,<br>114-120 |
| P,X  | WO 99 63096 A (LASSNER MICHAEL W<br>;RUEZINSKY DIANE M (US); CALGENE LLC (US))<br>9 December 1999 (1999-12-09)<br><br>claim 4; figure 1<br>& DATABASE GENSEQ [Online]<br>Derwent<br>accession no. Z45371,<br>27 March 2000 (2000-03-27)<br>see sequence<br>---  | 1-4,6,7,<br>20-31,<br>33-50,<br>54-61,<br>63-77,<br>79-100,<br>102-112,<br>114-120 |
| A  | FRENTZEN M (REPRINT): "Acyltransferases<br>from basic science to modified seed oils"<br>FETT - LIPID, WILEY-VCH VERLAG, WEINHEIM, DE,<br>vol. 100, no. 4/05, May 1998 (1998-05),<br>pages 161-166, XP002122744<br>ISSN: 0931-5985<br>the whole document<br>---  |  |
| A  | TANIYAMA YOSHIO ET AL: "Cloning and<br>expression of a novel lysophospholipase<br>which structurally resembles lecithin<br>cholesterol acyltransferase."<br>BIOCHEMICAL AND BIOPHYSICAL RESEARCH<br>COMMUNICATIONS,<br>vol. 257, no. 1,<br>2 April 1999 (1999-04-02), pages 50-56,<br>XP002161572<br>ISSN: 0006-291X<br>abstract<br>----- |  |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/23863

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-3(part)4(all),6-7,20-31,33-50,54-61,63-77,79-100,102-112,114-120(all part)

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-3(part)4 (all),6-7,20-31,33-50,54-61,63-77,  
79-100,102-112,114-120(all part)

SEQ ID 42 and related subject matter.

2. Claims: 1-3,5-120

Groups 2 through 37 - SEQ IDs 2-75 as listed in claim 5 and  
related subject-matter.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern'l application No

PCT/US 00/23863

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|--|------------------|-------------------------|------------------|
| WO 9963096 A                           | 09-12-1999 EP    | 1084256 A               | 21-03-2001       |

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